University of Agronomic Sciences
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Faculty of Agriculture

SCIENTIFIC PAPERS
SERIES A. AGRONOMY

Volume LXVIII, No. 1



2025
BUCHAREST

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VOLUME LXVIII, NO. 1, 2025

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PUBLISHER: University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Agriculture, Romania
Address: 59 Mărăşti Blvd, District 1, 011464, Bucharest, Romania
Phone/Fax: + 40 213 318-0466; E-mail: agronomyjournal@usamv.ro; Webpage: http://agronomyjournal.usamv.ro

CERES Publishing House
Address: 106 Izbiceni Street, District 1, Bucharest, Romania; E-mail: edituraceres@yahoo.com

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To be cited: Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025

The publisher is not responsible for the opinions published in the Volume. They represent the authors' point of view.

ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

International Database Indexing: Web of Science Core Collection (Emerging Sources Citation Index), CABI, Index Copernicus, Google Scholar, CNCSIS B+, Ulrich's Periodicals Directory, Research Bible, Scipio, Scientific Indexing Service, PBN (Polish Scholarly Bibliography), OCLC (WorldCat)

SUMMARY

SOIL SCIENCES

1. STUDY ON LAND AFFECTED BY EROSION IN GORJ COUNTY - **Mihaela BĂLAN, Oana Alina NIȚU** 17
2. THE INFLUENCE OF SOIL TILLAGE AND FERTILIZATION ON SOME QUALITY PARAMETERS OF GRAIN SORGHUM PRODUCTION UNDER THE CLIMATIC CONDITIONS OF THE SĂRĂȚENI-IALOMIȚA - **Andrei BĂNICĂ, Doru Ioan MARIN** 25
3. SOIL PHYSICAL PROPERTIES, SPECTRAL RESPONSE AND YIELD OF THE SUNFLOWER UNDER DIFFERENT TILLAGE SYSTEMS AT EZARENI FARM, IASI COUNTY, ROMANIA - **Serginho CAKPO, Gabriel Dumitru MIHU, Tudor George AOSTĂCIOAEI, Cosmin GHELBERE, Cosmin MOLOCEA, Gerard JITĂREANU, Denis ȚOPA** 30
4. RESEARCH ON THE INVENTORY OF THE MAIN PHYSICAL-HYDRIC AND CHEMICAL PROPERTIES OF THE MOLLIC PSAMOSOL IN THE SOUTHERN AREA OF OLTENIA (LEFT OF THE JIU RIVER) - **Jenica CĂLINA, Aurel CĂLINA, Marius MILUȚ** 43
5. BEHAVIOR OF WHEAT UNDER DIFFERENT TILLAGE SYSTEMS IN THE PEDOCLIMATIC CONDITIONS OF THE TRANSYLVANIAN PLATEAU - **Felicia CHEȚAN, Cornel CHEȚAN, Teodor RUSU, Alina ȘIMON, Camelia URDĂ, Alin POPA, Marius BĂRDAȘ, Adrian CECLAN, Ioan GAGA** 51
6. ORGANIC AGRICULTURE HAS DEFECTS IN THE SCOPE OF MINERAL FERTILIZATION - **Ali COSKAN** 59
7. A COMPARATIVE STUDY OF THE BENTONITES AND NATURAL ZEOLITES CAPACITIES TO REDUCE THE BIOAVAILABILITY OF HEAVY METALS IN A CONTAMINATED SOIL - **Mihaela COSTEA, Nicoleta-Olimpia VRÎNCEANU, Dumitru-Marian MOTELICĂ, Veronica TĂNASE, Mirela Alina SANDU, Costică CIONTU**..... 65
8. THE FUNCTIONAL MICROBIAL PROFILE IN HEAVY METAL CONTAMINATED SOILS AFTER ONE AND A HALF YEAR OF BIOREMEDIATION - **Loredana CRIȘAN, Vlad STOIAN, Larisa CORCOZ, Bianca POP, Alexandra GHEORGHIȚĂ, Anca PLEȘA, Roxana VIDICAN** 73
9. THE BEHAVIOR OF WINTER WHEAT IN THE CONSERVATIVE TILLAGE SYSTEM UNDER THE CONDITIONS AT A.R.D.S. PITEȘTI-ALBOTA - **Ilie Cătălin DINUȚĂ, Maria Magdalena PODEA, Doru Ioan MARIN** 80
10. REGARDING TRANSFER FACTOR OF SOIL POLLUTION WITH HEAVY METALS IN SOIL-PLANT SYSTEM - **Claudiu-Denis FILIP, Mirela Ana COMAN** 90
11. THE IMPACT OF DCD AND DMPP ON NITRIFICATION IN ALKALINE AND SILTY LOAM TEXTURED SOIL - **Murat GENCER, Mustafa GÖK** 100

12. THE EVOLUTION OF MICROBIAL FUNCTIONAL PROFILE INVOLVED IN DECOMPOSITION PROCESSES IN LONG-TERM FERTILIZED EXPERIMENTS - **Alexandra GHEORGHIȚĂ, Larisa CORCOZ, Bianca POP, Anca PLEȘA, Vlad STOIAN, Roxana VIDICAN** …… 108
13. AGROGENETIC EVOLUTION OF ARABLE CHERONEZOMS IN THE AREA BETWEEN THE PRUT AND NISTER RIVER: STAGES, TRENDS - **Gheorghe JIGĂU, Sergiu DOBROJAN, Galina DOBROJAN, Iulian JIGĂU** …… 120
14. MICROBIOLOGICAL DIVERSITY AND CONTROL OF SOILS AND WATERS FROM FOREST AND AGROECOSYSTEMS UNDER DIFFERENT HYDROTHERMAL SOIL REGIMES AND AS FACTORS FOR HIGH-QUALITY AND SAFE COMPOSTING - **Boyka MALCHEVA, Rozalina KOLEVA, Bilyana GRIGOROVA-PESHEVA, Pavel PAVLOV** …… 129
15. STUDY ON LAND SUITABILITY IN BUCU AREA, IALOMITA COUNTY, FOR FORESTRY USE - **Marian MUȘAT, Valentina Mihaela VASILE, Costel DOLOCAN, Georgian ARGATU** …… 138
16. SUSTAINABLE USE AND MANAGEMENT OF SOIL RESOURCES IN THE ALMĂJULUI VALLEY AREA, COUNTY CARAȘ-SEVERIN - **Lucian Dumitru NIȚĂ, Simona NIȚĂ, Karel Iaroslav LAȚO, Anișoara Duma COPCEA, Ioana-Alina HÎNDA, Alina LAȚO** …. 146
17. SOIL NUTRITIONAL STATUS FUNCTION OF BIOMASS HARVESTING IN NORTHWESTERN ROMANIA - **Dragoș Răzvan OROIAN, Petru BURDUHOS, Ioan OROIAN, Ioan PĂCURAR** …… 153
18. IMPACT OF DIGESTATE AND COMPOST APPLICATIONS ON SOIL CHEMICAL CHARACTERISTICS AND METALS AVAILABILITY IN A CONTAMINATED SOIL - **Florența PARASCHIV (JAFRI), Mihaela COSTEA, Nicoleta-Olimpia VRÎNCEANU, Veronica TĂNASE** …… 159
19. DYNAMICS OF FUNCTIONAL MICROBIOME IN POLLUTED SOIL AFTER ONE YEAR OF BIOREMEDIATION - **Bianca POP, Larisa CORCOZ, Alexandra GHEORGHIȚĂ, Anca PLEȘA, Vlad STOIAN, Roxana VIDICAN** …… 171
20. RESEARCH ON THE INFLUENCE OF ORGANIC AND MINERAL FERTILIZATION ON THE PHYSICAL PROPERTIES OF THE SOIL IN COVASNA COUNTY - **Bianca-Anamaria POPA, Mircea MIHALACHE** …… 181
21. IDENTIFICATION AND CHARACTERIZATION OF SOIL RESOURCES SPECIFIC TO THE FLOODPLAIN AREA OF ROSEȚI COMMUNE, CĂLĂRAȘI COUNTY - **Larisa Elena RÎPA (TOPÂRCEANU), Viorel GHIORȚAN, Georgeta GUȚĂ, Leonard ILIE** 187
22. DECREASE IN THE ACTIVITY OF SOIL FUNCTIONAL MICROBIOMES IN HEAVY METAL POLLUTED SOILS - **Vlad STOIAN, Roxana VIDICAN, Larisa CORCOZ, Anca PLEȘA, Alexandra GHEORGHIȚĂ, Bianca POP** …… 197
23. ASSESSMENT OF MICRO AND MACROELEMENTS UPTAKE IN MAIZE PLANTS IN RESPONSE TO DIFFERENT NITROGEN AND PHOSPHORUS FERTILIZATION RATES - **Veronica TĂNASE, Nicoleta Olimpia VRÎNCEANU, Mihaela PREDA, Mihaela COSTEA, Patrick URSAN** …… 204
24. SOIL PHOSPHOMONOSESTERASES ACTIVITY AND PHOSPHORUS AVAILABILITY IN DIFFERENT TILLAGE SYSTEMS AND INOCULATIONS WITH *Bacillus megaterium* var. *phosphaticum* - **Ana URSU, Geanina BIREESCU, Irina Gabriela CARA, Mariana RUSU, Denis ȚOPA, Gerard JITĂREANU** …… 214

25. INCREASED ACTIVITY OF SPECIALIZED FUNCTIONAL MICROBIOMES IN HISTORICALLY POLLUTED SOILS AFTER BIOREMEDIATION - **Roxana VIDICAN, Vlad STOIAN, Larisa CORCOZ, Anca PLEȘA, Bianca POP, Alexandra GHEORGHIȚĂ** …… 225

CROP SCIENCES

1. EFFECTS OF SOME BIOSTIMULATORS AT TWO POTATO CULTIVARS CULTIVATED AT DIFFERENT PLANT DENSITIES - **Lorena ADAM, Manuela HERMEZIU, Viorel ION** ……. 235
2. SUNFLOWER PRODUCTION OF SOME GENOTYPES IN YEARS 2022, 2023 AND 2024, IN ROMANIA - **Florin Gabriel ANTON, Maria JOIȚA-PĂCUREANU, Laura CONTESCU, Mihaela CERGAN, Elena PARTAL, Sabina PINTILIA** ……..…… 246
3. TILLAGE AND NITROGEN RATE EFFECTS ON MAIZE IN THE SPECIFIC GROWING CONDITIONS OF DANUBE MEADOW - **Albert AVARVAREI, Maria TOADER, Viorel ION** ……..……...…..…… 254
4. THE IMPACT OF PEDOCLIMATIC CONDITIONS ON THE PRODUCTION OF ESSENTIAL OIL AND LAVENDER HYDROLATE - **Roxana BĂLĂȘOIU (JIGĂU), Ilinca IMBREA, Laura ȘMULEAC, Diana OBISTIOIU, Ana-Mariana DÎNCU, Raul PAȘCALĂU, Florin IMBREA** ……..… 261
5. INFLUENCE OF CLIMATIC CONDITIONS ON POTATO TUBERS QUALITY - **Nina BĂRĂSCU, Manuela HERMEZIU, Anca-Camelia URDĂ, Lorena ADAM** 268
6. MODERN MAIZE CULTIVATION TECHNOLOGIES IN THE CONTEXT OF CLIMATIC CHALLENGES - **Lucian Florin BOTOȘ, Ioana-Alina HÎNDA, Florin IMBREA, Simona NIȚĂ, Lucian Dumitru NIȚĂ, Ștefan-Laurențiu BĂTRÎNA** ……..…..… 275
7. INFLUENCE OF TILLAGE AND VARIETY ON YIELD AND QUALITY OF CHICKPEA CROP ON THE REDDISH PRELUVOSOL FROM MOARA DOMNEASCĂ, ROMANIA - **Valentin-Marius CIONTU, Marga GRĂDILĂ, Daniel JALOBĂ, Mihai GÎDEA** ……..… 282
8. SOME *Asteraceae* SPECIES (*Asteraceae Martinov*) IN THE COLLECTION OF "ALEXANDRU CIUBOTARU" NATIONAL BOTANICAL GARDEN (INSTITUTE) AS POTENTIAL HONEY PLANTS - **Natalia CÎRLIG** ……..…… 289
9. EVALUATION OF QUANTITATIVE AND QUALITATIVE INDICATORS IN MAIZE HYBRIDS (*Zea mays* L.) CULTIVATED IN NORTH-EASTERN OF BULGARIA - **Vanya DELIBALTOVA** ……..……..…… 296
10. SCREENING OF BARBED GOATGRASS (*Aegilops triuncialis* L.) FOR NaCl SALINITY STRESS AT GERMINATION AND EARLY STAGES OF PLANT GROWTHT - **Gergana DESHEVA, Manol DESHEV, Evgeniya VALCHINOVA, Albena PENCHEVA, Bozhidar KYOSEV** ……..…..…… 304
11. YIELD STABILITY AND ITS ELEMENTS IN COMMON WINTER WHEAT VARIETIES UNDER PAZARDZHIK REGION CONDITIONS - **Evgeniy DIMITROV, Hristina NEDEVA, Rangel DRAGOV, Teodora ANGELOVA, Zlatina UHR, Blagoy ANDONOV** 311
12. GENETIC NATURE OF QUANTITATIVE TRAITS IN DURUM WHEAT - **Rangel DRAGOV, Krasimira TANEVA, Boryana HADZHIIVANOVA, Maria VIDEVA, Boyana TODOROVA, Rositsa CHOLAKOVA** ……..…… 320

13. EVALUATION OF NEW VIRGINIA TOBACCO GENOTYPES FOR YIELD, MORPHOLOGICAL AND CHEMICAL TRAITS - **Marina DRUMEVA-YONCHEVA, Neli KERANOVA, Yonko YONCHEV** 327
14. THE QUALITATIVE POTENTIAL OF SOME GRASS-LEGUME MIXTURES FOR FEED. CASE OF STUDY - **Carmen Claudia DURĂU, Codruța CHIȘ, Nicolae Marinel HORABLAGA, Christianna Maria ISTRATE-SCHILLER, Casiana Doina MIHUȚ, Vlad Dragoslav MIRCOV, Anișoara Claudia DUMA COPCEA, Daniela SCEDEI .**..... 336
15. COMPARATIVE TESTING OF SORGHUM, SUDANGRASS AND MAIZE SOWN AS A SECOND CROP AFTER WHEAT AND FALLOW PREDECESSORS - **Stanimir ENCHEV, Velimir DONCHEV** 342
16. EFFICACY OF BIOLOGICAL AND CHEMICAL SEED TREATMENTS FOR SUSTAINABLE MAIZE CROP PROTECTION **- Viorel FĂTU, Laura ȘOPTEREAN, Luxița RÎȘNOVEANU, Mihaela Monica DINU, Ana-Cristina FĂTU** 349
17. ON THE GROWTH AND DEVELOPMENT PROCESS OF GRASS MIXTURES AND LEGUMES FOR RIPARIAN GRASSLAND RENOVATION - **Adrian FILIP, Niculae DINCĂ, Ana-Maria STANCIU, Gabriela-Cristina MANTEA, Daniel DUNEA** 357
18. THE WESTERN CORN ROOTWORM (*Diabrotica virgifera virgifera* Le Conte) POPULATION IS INCREASING IN THE SOUTHEAST OF ROMANIA - **Emil GEORGESCU, Maria TOADER, Lidia CANĂ, Elena PARTAL, Marius BORDEI, Mali Sanda MANOLE** 363
19. INTEGRATION AND FUNCTIONALITY OF IPM STRATEGIES FOCUSING ON INSECT PESTS IN VARIOUS CROPS - **Ioana GROZEA, Diana-Maria PURICE, Ramona ȘTEF, Alin CARABET, Adrian GROZEA, Ana-Maria VIRTEIU** 371
20. EFFECTS OF DIFFERENT NITROGEN FERTILIZER RELEASE TYPES AND APPLICATION MOMENTS IN HYBRID SEED MAIZE PRODUCTION - **Lucian Constantin HARAGA, Lenuța Iuliana EPURE, Viorel ION** 379
21. WHEAT FLAG LEAF MORPHOANATOMICAL CHARACTERISTICS AND GRAIN YIELD COMPONENTS UNDER FIELD CONDITIONS - **Florența-Elena HELEPCIUC, Leonard-Alexandru DUMITRU, Alina Gabriela TURCU, Alexandra Gabriela CIOCAN, Daniel CRISTINA, Matilda CIUCĂ, Elena PETCU, Cătălin LAZĂR, Elena Monica MITOI** 393
22. IMPACT OF WEATHER PARAMETERS AND THE FUNGICIDE SPRAYING PROGRAM ON THE LATE BLIGHT (*Phytophthora infestans*) OF POTATO IN BRASOV AREA - **Manuela HERMEZIU, Lorena ADAM** 402
23. STUDY REGARDING THE INFLUENCE OF NITRIC, AMMONIACAL NITROGEN FERTILIZATION AND VARIETY ON WINTER WHEAT (*Triticum aestivum* L.) PROTEIN CONTENT - **Denisa Cristiana HETEA, Simona NIȚĂ, Ștefan-Laurențiu BĂTRÎNA, Ioana-Alina HÎNDA, Piotr PRUS, Florin IMBREA** 408
24. *Carthamus tinctorius* L. – A SPECIES WITH POTENTIAL FOR EXPANDING CULTIVATED AREAS - **Ioana-Alina HÎNDA, Florin IMBREA, Simona NIȚĂ, Lucian Florin BOTOȘ, Georgeta POP, Ștefan-Laurențiu BĂTRÎNA** 419
25. VARIABILITY OF MORPHOLOGICAL TRAITS IN ROMANIAN WILD THYME POPULATIONS - **Ilinca Merima IMBREA, Rodica BEICU, Sorina POPESCU, Georgeta POP, Alina NEACȘU, Anca HULEA, Mariana HANCZIG, Mihai HERBEI** 427

26. NEW ASPECTS WITH WEED COMPETITION AND CONTROL FROM THE SUNFLOWER CROP - **Nicolaie IONESCU, Diana Maria POPESCU, Mariana Cristina NICOLAE, Cristina GHIORGHE, Maria Magdalena PODEA, Ilie Cătălin DINUȚĂ, Robert Marian GHEORGHE** 436
27. MONITORING OF *Rhagoletis cerasi* WITH THE HELP OF DECIS TRAP TRAPS AND MONILIOSIS IN THE CHERRY ORCHARD FROM USAMV OF BUCHAREST **- Rada ISTRATE, Cristinel Relu ZALĂ, Mali-Sanda MANOLE** 443
28. RESULTS OF THE COMPARATIVE RESEARCH ON DISEASES ASSOCIATED WITH INVASIVE NEMATODES IN MAIZE PLANTATIONS UNDER THE CONDITIONS OF THE REPUBLIC OF MOLDOVA - **Elena IURCU-STRAISTARU, Alexei BIVOL, Natalia CÎRLIG, Ștefan RUSU** 451
29. EFFECT OF LEGUME-BARLEY INTERCROPPING ON POPULATION DYNAMICS OF CEREAL APHIDS IN DIFFERENT BARLEY VARIETIES - **Lilyana KOLEVA, Georgi DIMITROV** 459
30. RESEARCH ON THE IMPACT OF NITROGEN AND PHOSPHORUS FERTILIZERS ON WINTER WHEAT YIELD AND QUALITY UNDER THE PEDOCLIMATIC CONDITIONS OF CENTRAL MOLDOVA - **Alexandra LEONTE, Simona Florina ISTICIOAIA, Andreea-Sabina PINTILIE, Lorena Diana POPA, Doru STANCIU, Paula Lucelia PINTILIE, Adina Cătălina DRUȚU, Nicoleta VRÎNCEANU** 465
31. RESEARCH ON MICROBIOTA ASSOCIATED WITH SUNFLOWER SEEDS - **Maria-Alexandra MARIN, Beatrice Michaela IACOMI, Emil GEORGESCU, Stelica CRISTEA** 471
32. CORRELATION OF SUNFLOWER YIELD WITH HEAD YIELDING ELEMENTS ACCORDING TO ROW SPACING AND PLANT DENSITY - **Vicorița MARIN, Lenuța Iuliana EPURE, Nicoleta ION, Viorel ION**..... 476
33. INFLUENCE OF ENVIRONMENTAL CONDITIONS ON RHEOLOGICAL PARAMETERS IN SEVERAL ROMANIAN WINTER WHEAT VARIETIES RELEASED BY NARDI FUNDULEA - **Cristina Mihaela MARINCIU, Cătălin LAZĂR, Gabriela ȘERBAN, Mihai TILIHOI, Gabriela PĂUNESCU, Cecilia BĂNĂȚEANU, Beniamin-Emanuel ANDRAȘ, Cristina MELUCĂ, Iustina LOBONTIU, Zsuzsa DOMOKOȘ, Simona Florina ISTICIOAIA, Andreea-Sabina PINTILIE, Andreea ENEA, Emanuela LUNGU, Cornelia TICAN, Nicolae SĂULESCU** 484
34. EVALUATION OF SOIL AND FOLIAR HERBICIDAL APPLICATIONS AT CAMELINA (*Camelina sativa* (L.) Crantz) - **Nesho NESHEV, Marina MARCHEVA** 490
35. INVESTIGATING THE IMPACT OF SPECIFIC TECHNOLOGIES ON THE PRODUCTION AND QUALITY OF THE AUTUMN BARLEY CROP - **Simona NIȚĂ, Ilinca Merima IMBREA, Lucian Dumitru NIȚĂ, Ioana-Alina HÎNDA, Ștefan-Laurențiu BĂTRÎNA, Denisa Cristiana HETEA, Lucian BOTOȘ** 503
36. RESEARCH ON THE INFLUENCE OF FERTILIZATION ON *Lavandula angustifolia* Mill. SPECIES IN THE ORGANIC CULTURE OF THE BUFTEA AREA (ILFOV) - **Gabriela OPREA (BUTNARIU), Monica Luminița BADEA, Adina NICHITA, Doru Ioan MARIN** 510
37. DENSITY-BASED ASSESSMENT OF *Adonis vernalis* ABUNDANCE IN NATIVE HABITATS - **Florin PĂCURAR, Ioan ROTAR, Roxana VIDICAN, Ioana VAIDA (GHEȚE), Anca PLEȘA, Vlad STOIAN, Liviu TOMOȘ, Alexandru GHEȚE** 516
38. INFLUENCE OF NITROGEN FERTILIZATION ON TWO-ROWED WINTER BARLEY GENOTYPES UNDER 2023-2024 YEAR IN SOUTHEAST OF ROMANIA - **Eugen PETCU, Liliana VASILESCU, Viorel ION** 524

39. EFFECT OF METEOROLOGICAL CONDITIONS ON THE PRODUCTIVITY OF MAIZE HYBRIDS OF DIFFERENT MATURITY GROUPS - **Nataliya PETROVSKA, Emil VASILEV, Viliana VASILEVA** 530
40. STUDY ON THE ADAPTABILITY OF SOME TRITICALE GENOTYPES TO DIFFERENT CLIMATE AND SOIL CONDITIONS - **Andreea-Sabina PINTILIE, Denisia-Mihaela IACOBUȚ, Alexandra LEONTE, Doru STANCIU, Paula Lucelia PINTILIE, Rozalia KADAR, Diana HIRIȘCĂU, Georgeta TRAȘCĂ, Cristina GHIORGHE, Marian-Robert GHEORGHE, Teodor ROBU** 536
41. SUGAR BEET PEST DYNAMICS IN THE CONDITIONS OF CENTRAL MOLDOVA (ROMANIA) - **Paula Lucelia PINTILIE, Roxana Georgiana AMARGHIOALEI, Elena TROTUȘ, Andreea-Sabina PINTILIE, Alexandra LEONTE, Simona Florina ISTICIOAIA, Andreea ENEA, Roxana ZAHARIA, Carmen MINCEA** 542
42. ASSESSMENT OF THE THERAPEUTIC POTENTIAL APPLICATION OF THE EXTRACTS FROM DIOECIOUS HEMP (*Cannabis sativa* L.) - **Georgeta POP, Ersilia Călina ALEXA, Ilinca Merima IMBREA, Nicolae-Marinel HORABLAGA, Diana OBIȘTIOIU** 548
43. TESTING THE EFFICACY OF ESSENTIAL OILS AGAINST *Drosophila suzukii* (Matsumura) - **Stefan RASHEV, Nedyalka PALAGACHEVA, Ivan ARABADJIEV, Stoyan GEORGIEV** 554
44. ASSESSMENT OF THE EFFECTS OF SLOW-RELEASE FERTILIZERS APPLICATION OVER THE AMOUNT OF NITROGEN LEACHED AND OF THE DEVELOPMENT OF WINTER WHEAT PLANTS IN CONTROLLED CLIMATE CONDITIONS - **Lucian RAUS, Diana BOLOHAN** 560
45. EFFICACY OF BIOSTIMULANTS AGAINST CABBAGE STEM FLEA BEETLES IN WINTER OILSEED RAPE UNDER FIELD CONDITIONS IN THE UK - **Hamad SAEED** 569
46. MODELING BIOLOGICAL GROWTH OF *Lolium perenne* SPECIES UNDER CURRENT CLIMATE CHANGE CONDITIONS - **Ionel SAMFIRA, Nicolae Marinel HORABLAGA, Ramona Loredana TOPORAN, Adina HORABLAGA, Saida FEIER DAVID, Cristian BOSTAN** 574
47. STUDY OF THE EFFECT OF SOME FOLIAR HERBICIDES ON ALFALFA (*Medicago sativa* L.) IN CHANGING CLIMATE CONDITIONS - **Atanas SEVOV, Nesho NESHEV, Mariyan YANEV, Anyo MITKOV** 583
48. STUDIES ON THE INFLUENCE OF GROWTH REGULATORS IN REDUCING THE EFFECTS OF CLIMATE CHANGE ON SOYBEAN CULTIVATION IN CENTRAL MOLDAVIA - **Doru STANCIU, Oana MÎRZAN, Margareta NAIE, Alexandra LEONTE, Andreea-Sabina PINTILIE, Denisia-Mihaela IACOBUȚ, Roxana-Georgiana AMARGHIOALEI, Cosmin-Alexandru MURARU, Carmenica-Doina JITĂREANU** 592
49. UNVEILING THE EFFECTS OF DROUGHT STRESS ON MAIZE HYBRIDS: CHANGES IN CHLOROPHYLL CONTENT, MORPHOLOGICAL TRAITS AND SOIL-PLANT WATER DYNAMICS - **Valentina Ancuța STOIAN, Anamaria VÂTCĂ, Mădălina TRUȘCĂ, Vlad STOIAN, Alin Laviniu POPA, Alina TOȘA, Ștefania GÂDEA, Roxana VIDICAN, Florin PĂCURAR, Sorin VÂTCĂ** 599
50. EFFECT OF FOLIAR FERTILIZATION ON PROTEIN LEVELS AND NUTRITIONAL VALUE OF THREE HYBRID MAIZE BIOMASS - **Antoniya STOYANOVA, Georgi STOYANOV, Gancho GANCHEV** 608
51. STUDIES ON THE CULTIVATION OF WHITE MUSTARD (*Sinapis alba*) UNDER THE AGROECOLOGICAL CONDITIONS OF SOUTHERN DOBROGEA – DELENI IN A CONVENTIONAL SYSTEM - **Ciprian Traian STROE, Liliana PANAITESCU** 616

52. QUINOA (*Chenopodium quinoa* Willd.): A PROMISING NEW CROP FOR ROMANIA - **Lizica SZILAGYI, Mihai GÎDEA** 625
53. IMPROVING SOYBEAN QUALITY PARAMETERS BY SOWING ON DIFFERENT DATES IN VARIOUS CLIMATIC YEARS - **Alina ŞIMON, Felicia CHEŢAN, Adrian CECLAN, Alin POPA, Camelia URDĂ, Nicolae TRITEAN, Raluca REZI, Florin RUSSU, Adrian NEGREA** 631
54. STUDY OF UNSTABLE PHENOTYPIC BEAN SEEDS - **Eliza TEODORESCU, Elena BARCANU, Ion GHERASE, Ovidia AGAPIE** 640
55. FUTURE VISIONS OF INTEGRATED PEST MANAGEMENT (IPM) FOR MAIZE CROPS IN ROMANIA - **Maria TOADER, Emil GEORGESCU, Cristina CIONGA, Cristina RADU, Viorel ION, Lenuţa Iuliana EPURE, Adrian Gheorghe BĂŞA, Mirela Elena DUŞA, Mihaela Valentina VASILE, Alina Maria IONESCU** 649
56. EVALUATION OF TALL FESCUE (*Festuca arundinacea*) GENETIC RESOURCES FOR BREEDING ACTIVITY - **Monica Alexandrina TOD, Mironela BĂLAN, Sorina NIŢU, Paul ZEVEDEI** 655
57. RELATIONSHIPS OF NDVI AND CHLOROPHYLL CONTENT WITH YIELD COMPONENTS OF DIFFERENT ACCESSIONS OF *Phaseolus vulgaris* L. AND VIGNA (*Vigna unguiculata* L. Walp.) - **Mima TODOROVA, Tsvetelina STOILOVA, Mariya GERDZHIKOVA, Hristina RANGELOVA, Zornitza ZHERKOVA, Neli GROZEVA, Milena TZANOVA** 661
58. THE EVALUATION OF FERTILIZATION SCHEMES OPTIMISATION AT TWO ROW BARLEY CROP IN ORDER TO INCREASE THE HARVEST PROCESSING VALUE - **Alexandru Iulian TOMA, Ricuţa Vasilica DOBRINOIU** 666
59. CRITICAL SALINITY THRESHOLDS IMPACTING WHEAT GERMINATION: DETERMINING DOSE-DEPENDENT RESPONSES ACROSS VARIETIES - **Mădălina TRUŞCĂ, Valentina STOIAN, Anamaria VÂTCĂ, Vlad STOIAN, Roxana VIDICAN, Florin PĂCURAR, Ştefania GÂDEA, Sorin VÂTCĂ** 673
60. DIVA-GIS APPROACH TO DIVERSITY ANALYSIS OF *Aegilops cylindrica* GERMPLASM - **Evgeniya VALCHINOVA, Manol DESHEV, Bozhidar KYOSEV, Albena PENCHEVA, Gergana DESHEVA** 683
61. GENETIC CONTROL AND COMBINING ABILITY IN LINE BY TESTER CROSSES OF COTTON - **Neli VALKOVA, Minka KOLEVA, Valentina DIMITROVA, Bogomil MIHAYLOV** 689
62. RESPONSE OF MAIZE BREEDING TO CLIMATE CHANGE - **Emil VASILEV, Nataliya PETROVSKA, Viliana VASILEVA** 700
63. EVALUATION OF FOLIAR HERBICIDES FOR WEED CONTROL IN MAIZE (*Zea mays* L.) - **Mariyan YANEV** 706
64. RELATIONSHIPS BETWEEN MORPHOMETRIC AND QUALITY PARAMETERS IN WHEAT GRAINS - **Volkan YEŞİL, Vali ABDİYEV, Özgür TATAR** 716
65. STUDY ON THE DEVELOPMENT AND YIELD OF SAFFRON CROCUS (*Crocus sativus* L.) IN ORGANIC FARMING CONDITIONS IN CENTRAL SOUTHERN BULGARIA - **Plamen ZOROVSKI** 722

MISCELLANEOUS

1. SURVEY ON SUSTAINABLE PROMOTION OF NUTRITIONAL STATUS OF CROPS - **Oana ABRUDAN (RADU), Camelia OROIAN, Claudia BALINT, Antonia ODAGIU** …. 731
2. A COMPARATIVE STUDY ON THE EVOLUTION OF PLANTED BLACK PINE SAPLINGS ON THE STERILE DUMPS FROM RECEA ȘUNCUIUȘ QUARRY, BIHOR COUNTY - **Bogdan BODEA, Cristian Mihai ENESCU, Ruben BUDĂU, Adrian Ioan TIMOFTE** …… 738
3. RESEARCH ON PRODUCTIVITY INDICES IN SOME DEMONSTRATION BATCHES OF AUTUMN WHEAT, AT SCDA BRAILA, IN THE LAST TWO AGRICULTURAL YEARS - **Marian BRĂILĂ, Daniela TRIFAN, Emanuela LUNGU, Mircea MIHALACHE** 746
4. HALOPHYTE BIOACTIVE COMPOUNDS: A REVIEW OF THEIR CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITIES - **Nicoleta-Olimpia CIOARĂ (ANDREI), Amalia Carmen MITELUȚ** …… 753
5. RECYCLING OF WASTEWATER FROM THE CULTIVATION OF *Spirulina platensis* THROUGH ITS USE AS A BIOSTIMULANT FOR THE GERMINATION OF *Phacelia tanacetifolia* Benth. (Melifera) SEEDS MAINTAINED IN COLLECTIONS - **Sergiu DOBROJAN, Galina DOBROJAN, Gheorghe JIGĂU, Gabriel Ionuț PLAVAN** ……. 765
6. SURVIVAL RATE OF OAK (*Quercus* L.) SEEDLINGS IN AN AGROFORESTRY SYSTEM ESTABLISH IN HORTINOVA NURSERY (CÂRCEA, DOLJ COUNTY) - **Cristian Mihai ENESCU** …… 771
7. TRACKING THE PERCENTAGE OF SEED GERMINATION WHEN THE BASIS FOR THE SPROUTS IS PURE COMPOST AND COMPOST WITH ADDITIVES - **Bilyana GRIGOROVA-PESHEVA, Boyka MALCHEVA, Asen PESHEV** …… 777
8. HARMFUL AND BENEFICIAL FAUNA ASSOCIATED WITH MEDICINAL AND AROMATIC PLANTS - **Minodora GUTUE, Ionela DOBRIN, Simona-Daniela MIHAI, Elena Loredana SFETCU, Fulvia-Florica VLAD** …… 784
9. BIOACTIVE POTENTIAL OF SPENT *Rosa damascena* Mill. PETALS EXTRACT: ANTIOXIDANT AND ANTIMICROBIAL PROPERTIES - **Nikolay KOLEV, Mihaela IVANOVA, Desislava VLAHOVA-VANGELOVA, Alexander BALABANOV, Yulian TUMBARSKI, Milena DIMITROVA-DICHEVA, Francesco VIZZARRI, Lubomir ONDRUSKA** …… 792
10. EVALUATION OF THE INHIBITORY EFFECT OF ESSENTIAL OILS AGAINST *Fusarium sporotrichioides* ISOLATED FOR THE FIRST TIME FROM STRAWBERRY PLANTS IN ROMANIA - **Maria-Cristina LUMÎNARE, Daniel-Nicolae COJANU, Lavinia-Diana-Nicoleta BUTURUGĂ BARBU, Oana-Alina BOIU-SICUIA, Stelica CRISTEA** …… 800
11. EXPERIMENTAL RESEARCH FOR THE PROMOTION OF TECHNICAL EQUIPMENT FOR CLEARING MOUNDS AND FERTILIZING SOILS OCCUPIED BY GRASSLANDS - **Eugen MARIN, Vasile MOCANU, Tudor-Adrian ENE, Dragoș MANEA, Laurențiu-Constantin VLĂDUȚOIU, Alexandru IONESCU, Gheorghe STROESCU** …… 810
12. METHODS FOR DETERMINING PROTEINS AND AMINO ACIDS AND THE IMPORTANCE OF THEIR USE FOR IDENTIFYING SUSTAINABLE SOURCES OF DIETARY PROTEINS. A REVIEW - **Gheorghe MATEI, Alina Mădălina PLEȘOIANU, Lorena Diana POPA, Simona Florina ISTICIOAIA, Valentin Nicolae VLĂDUȚ** …… 816

13. PARTICULARITIES REGARDING THE TENDING OPERATIONS IN FOREST SHELTERBELTS - **Elena MIHĂILĂ, Mihăiță BÎTCĂ, Adrian TUDORA, Dorina DRĂGAN, Elena ACHIM, Any Mary PETRIȚAN** ……..…….……..…… 828
14. METHODOLOGY AND EVALUATION CRITERIA OF THE CONSERVATION STATUS OF GRASSLAND HABITATS OF COMMUNITY INTEREST IN ROMANIA **- Simona MIHĂILESCU, Ioana VICOL, Marilena ONETE, Luiza-Silvia MIHAI, Tiberiu SAHLEAN** ……..…….……..…… 836
15. POTENTIAL HABITAT PREDICTION FOR THE CURRENT DISTRIBUTION OF *Tozzia alpina* subsp. *carpathica* IN THE ROMANIAN CARPATHIANS - **Georgiana-Roxana NICOARĂ, Florian-Paul BODESCU, Marilena ONETE, Simona MIHĂILESCU** …… 846
16. STRUCTURAL AND FLORISTIC DIVERSITY OF THE SHRUBLANDS VEGETATION IN THE CĂPĂȚÂNII MOUNTAINS, ROMANIAN CARPATHIANS - **Mariana NICULESCU** ……..…… 856
17. ROLE ON THE PRODUCTIVITY AND NUTRACEUTICAL PROPERTIES OF CORN BY NITROGEN-FIXING AND PHOSPHORUS-SOLUBILIZING COMPONENT OF THE SOIL MICROBIOME - **Luca PATRUNO, Veronica SĂRĂȚEANU** ……..……. 869
18. ASSESSMENT OF ANTIFUNGAL AND ANTIBACTERIAL ACTIVITY OF CINNAMON AND CLOVE ESSENTIAL OILS - **Paul-Alexandru POPESCU, Wahauwouélé Hermann COULIBALY, Yabo Majoie Géroxie TOHOYESSOU, Elisabeta Elena POPA, Mihaela GEICU-CRISTEA, Mihaela Cristina DRĂGHICI, Amalia Carmen MITELUȚ, Mona Elena POPA** ……..……. 878
19. GLIMPSES INTO THE BIODIVERSITY OF SFÂNTU GHEORGHE, TULCEA COUNTY - **Mala-Maria STAVRESCU-BEDIVAN, Emilia Brîndușa SĂNDULESCU, Ionela DOBRIN, Elena Loredana SFETCU, Cristina Florentina ALISTAR**……..……..... 885
20. SOME AGROBIOLOGICAL FEATURES AND QUALITY INDICES OF THE BIOMASS OF *Panicum virgatum* and P*anicum miliaceum* GROWN UNDER THE CONDITIONS OF THE REPUBLIC OF MOLDOVA - **Victor ȚÎȚEI** ……..…….……..…… 892

SOIL SCIENCES

Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

STUDY ON LAND AFFECTED BY EROSION IN GORJ COUNTY

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Abstract

In Gorj County, water erosion is the most common form of soil degradation, affecting an area of 139,027.95 ha, i.e. approximately 57.04% of the total agricultural land. Of these, 134,940.26 ha are affected by surface erosion, and 4,087.69 ha by depth erosion. The diversity of the relief, anthropogenic productive activities and climate change contribute to the extension of these erosion processes, significantly affecting agricultural productivity on the affected areas. Soil erosion in Gorj is thus a major problem for the agricultural sustainability of the region. In the internal hollow, more precisely in the secondary hollows of Tismana, Peştişani, Crasna, Novaci, Polovragi, there are soils evolved on fluvial gravels with a strong acidic character, brought from the mountains. These soils contain a high percentage of skeletal material, which acts as a limiting factor by reducing the edaphic volume of the soil. In these areas, the land has slopes greater than 5%, and surface erosion occurs over the following areas: 7,793.52 hectares in Padeș, 5,733.88 hectares in Crasna, 4,194.32 hectares in Tismana, 3,824.80 hectares in Novaci, and 1,971.58 hectares in Polovragi.

Key words*: surface erosion, depth erosion, soil, slope, hollow.*

INTRODUCTION

The new EU Soils Strategy underlines the importance of healthy soils in achieving climate and biodiversity goals, with the aim of protecting the soil, reducing erosion, increasing organic matter, restoring degraded soils in the context of identified threats, such as erosion, flooding and loss of soil biodiversity (Mușat et al., 2021; EC Law, 2021). Among the processes that degrade soil quality, erosion is a particularly important factor, both in terms of the damage it causes and the affected areas (Bălan et al., 2024).

Depending on the intensity with which it manifests itself, but also on the consequences it leaves on the soil, erosion can be surface or in depth.

Erosion causes a significant decrease in soil fertility, as this process removes fertile layers from the upper horizons, which contain a significant amount of organic matter and nutrients (Bălan et al., 2024).

Also, runoff generated by erosion reduces crop yields, disrupts the soil water regime (Corcheș, 2023; Nițu et al., 2023) and constitutes a major mechanism for the transport of chemical pollutants in the river network.

Gorj County, located in south-western part of Romania, in the north of Oltenia and on the middle course of the Jiu River, is distinguished by a remarkable geographical diversity, which includes mountainous, submontane, hilly and plain areas. This variability of the landscape, correlated with the climatic conditions specific to the region, makes the soils in the county susceptible to erosion, a natural process of degradation that significantly affects the quality and fertility of agricultural land.

Soil erosion is one of the most serious environmental and agricultural problems in Gorj County, having a significant impact on natural resources, biodiversity and the local economy. Natural factors, such as heavy rainfall, steep slopes and lack of adequate vegetation, contribute to the acceleration of this phenomenon (Calina et al., 2021). At the same time, human activities, such as deforestation, mining and land misuse, amplify the effects of erosion, endangering large areas of agricultural land and forests.

In Gorj County, erosion is the main form of soil degradation, affecting approximately 139,027.95 ha, which represents 57.04% of the total agricultural area. This phenomenon contributes significantly to the reduction of agricultural production, in some cases even to the halving of crop yields, and in this county,

agriculture and animal husbandry are significant sectors in the local economy, primarily for sustaining the standard of living of the local population. Introducing good soil protection plants in the crop, with an appropriate productive capacity regardless of environmental conditions, has significant consequences on soil stability (Iancu et al., 2009). At the same time, the organic and mineral fertilizers used annually on soils affected by erosion, which have damaged physical and chemical properties, exert a positive influence on both the plants grown on these lands and on the soils (Iancu et al., 2009). It can therefore be said, in this regard, that the genotype-environment relationship can be considered an important indicator in setting limits for the use of agro-technical factors, aiming to increase production and maintaining soil quality (Iancu et al., 2021).

Gorj County faces a variety of forms of soil degradation, which emphasizes the importance of sustainable management of natural resources. The impact of soil erosion is significant, both on local ecosystems and on human communities. The loss of fertile soil reduces agricultural productivity, destabilizes ecosystems, and can lead to landslides, affecting infrastructure and housing in vulnerable areas (Kalmar et al., 2022). At the same time, excessive sedimentation of watercourses can have negative effects on the quality of water resources and biodiversity.

In this context, Gorj County is a relevant example for understanding the challenges related to sustainable soil management in regions affected by erosion. This phenomenon requires the implementation of effective prevention and control measures to limit soil degradation (Calina et al., 2021; Bouma et al., 2017; Davidson, 2000; Rhodes, 2017; Smith, 2012; Várallyay, 2010) and to rehabilitate them, by changing production technologies, to improve physical, chemical and biological properties, as well as maintaining a healthy environment (Popescu & Bălan; 2024; Zafiu & Mihalache, 2021).

MATERIALS AND METHODS

This study was carried out in Gorj County, a county that, due to the specificity of its geographical location, benefits from natural conditions that have favoured the manifestation of the water erosion process and it is aimed to identify the surfaces affected by slow surface geological erosion, as well as the ones affected by depth erosion.

For this study, field and laboratory research was carried out. During the research, the raw results obtained in the field and laboratory phases were processed and interpreted in the office phase.

In addition, simultaneously, the statistical data and documents existing at O.S.P.A. Gorj (Archive of O.S.P.A. Gorj, 1979-2010, 1980-2010, 2010, 2018) were studied and analysed, in order to highlight the areas affected by erosion in the county, but a review of the specialized literature was also made, in this regard, in order to provide a better understanding of this phenomenon, as well as its intensity.

At the same time, the study also focused on identifying the main measures that could be taken to prevent soil erosion and restore degraded land.

RESULTS AND DISCUSSIONS

Erosion is a particularly complex physical and physical chemical process, which consists in the breakdown of structural aggregates even up to the phases of elementary particles and their gravitational transport by the action of water or wind.

Erosion is influenced by the slope in the sense that it manifests itself with a greater intensity on sloping lands. At the same time, erosion is also influenced by the degree in which the land is covered by vegetation, in the sense that when the soil has a lower degree of vegetation cover, erosion manifests itself with a higher intensity. Erosion is also greatly influenced by the anthropogenic factor, through negative actions (taking into cultivation the land with a very high slope, executing the works from the hill to the valley or on the line of the highest slope, dividing and orienting them with the long side in the direction of the greatest slope or perpendicular to the contour lines, cultivation of ploughs on very steep land, clearing pastures, irrational deforestation of forests, etc.) that it undertakes.

Thus, in Gorj County, erosion is caused by intense rainfall, frequent in this region, which generates a large amount of runoff that

destabilizes the soil, the lack of vegetation on steep slopes, either due to natural causes or due to deforestation, which aggravates erosion and, last but not least, the geological characteristics of the soil, which in certain areas are poorly consolidated, allowing quick washing of soil particles. Also, human activities such as massive deforestation for timber and the expansion of agricultural areas reduce the soil's ability to retain water and expose it directly to erosion processes. Mining, such as lignite in the Rovinari-Motru basin, disrupts the natural balance of the land, generating severe erosion and pollution, but also inappropriate agricultural practices, such as cultivating land on a steep slope, contribute to the loss of the fertile soil layer.

Of the total agricultural area of 243,768 ha, surface erosion is manifested on 134,940.26 ha, representing 55.36%, and deep erosion 4,087.69 ha, which represents approximately 1.68% of the agricultural area of the county (Figure 1).

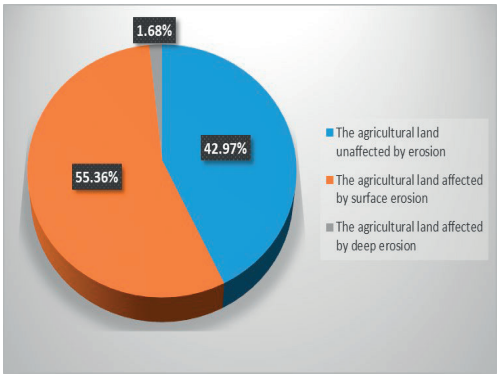


Figure 1. Structure of the agricultural area according to the degree of erosion damage (%)

In Gorj County, water erosion is the most widespread form of soil degradation and affects an area of 139,027.95 ha, of which surface erosion occurs on 134,940.26 ha representing 97.06% of the eroded area, and depth erosion occurs on 4,087.69 ha, which represents 2.94% of the area affected by erosion.

It can therefore be said that water erosion is the most complex, serious and extensive form of soil degradation in Gorj County, strongly affecting soil properties and its production capacity (Bălan & Popescu, 2024).

Surface erosion is very harmful because it contributes to the removal of the upper horizons of the soil, horizons in which humus and nutrients are accumulated, where intense microbiological activity takes place; the upper horizons also have the most favourable physical and hydro physical properties (Muşat et al., 2023).

The danger of surface erosion also lies in the fact that it is more difficult to observe and is not given due importance because its effects are felt especially after the small productions obtained and most of the time the low productions are attributed to other causes.

Surface erosion occurs under the destructive action of raindrops and/or dispersed runoff, lamination or in the form of small trickles or streams (Figure 2).

The water droplets and the dispersed runoff in the form of a uniform layer of water, acting on the surface of the slopes on which the precipitation falls, produce a fairly uniform erosion of the soil, which becomes evident even for non-specialists when reaching the lower horizons of the soil observable due to different colours from the upper horizon.



Figure 2. Surface erosion - Trickles in Novaci-Rînca area, Gorj County

Table 1 shows the surface erosion on administrative territorial units and on degrees of erosion.

From this table it can be seen that the territorial administrative units most affected by surface erosion are: Padeş with 7,793.52 ha, Crasna with 5,733.88, Tismana with 4,194.32 ha, Runcu with 3,873.60 ha, Novaci with 3,824.80 ha and Baia de Fier with 3,653.78 ha. This can be explained by the fact that within these ATUs there are soils evolved on fluvial gravels with a strong acidic

character, brought from the mountains, soils that contain a high percentage of skeletal material, which manifests itself as a limiting factor by decreasing the soil volume, which gives them a lower resistance to the erosion process.

Thus, due to the predominantly mountainous and hilly terrain, as well as frequent rainfall, ATU Padeş is the area most affected by surface erosion. The strong erosion manifests itself on an area of 4,099.92 ha, which represents 52.6% of the total area affected by erosion, while the very strong erosion, affects an area of 2,024.62 ha, which means 25.98%. Thus, it can be concluded that strong and very strong surface erosion manifests itself on 79.58% of the total area affected by erosion.

Furthermore, the Crasna administrative-territorial unit (ATU) is located in the sub-Carpathian hills and mountains area, with a varied terrain covered by forests, pastures but also by agricultural land in the lower areas, is the second most affected ATU by surface erosion. From the total affected area of 5,733.88 ha, strong, very strong and excessive surface erosion manifests itself on 3,016.48 ha, which represents 52.60% of the area affected.

Regarding the ATU Tismana, out of the total area of 4,194.32 ha affected by erosion, the strong, very strong and excessive erosion is manifested on 59.08% of the surface, that means on 2,477.92 ha.

Table 1. Surface erosion on ATUs and on erosion degrees in Gorj county

No. crt.	Administrative-Territorial Unit	Eroded area (ha)	Degree of erosion				
			Mild erosion	Moderate	Strong	Very strong	Excessive
0	1	2	3	4	5	6	7
1	Padeş	7,793.52	468.10	877.20	4,099.92	2,024.62	323.68
2	Crasna	5,733.88	1,459.80	1,257.60	1,458.50	1,125.90	432.08
3	Tismana	4,194.32	881.60	834.80	1,417.92	720.00	340.00
4	Runcu	3,873.60	422.00	1,068.40	1,548.00	625.10	210.10
5	Novaci	3,824.80	591.30	708.90	1,539.30	780.30	205.00
6	Baia de Fier	3,653.78	504.90	445.20	1,780.00	620.00	303.68
7	Bălăneşti	3,446.21	314.00	931.00	1,431.21	590.00	180.00
8	Bustuchin	3,315.40	424.40	790.60	549.90	1,202.50	348.00
9	Peştişani	3,256.40	348.10	1,287.70	1,000.30	460.20	160.10
10	Cruşet	3,062.31	505.38	530.28	1,066.45	830.00	130.20
11	Borăscu	3,046.45	536.90	720.51	780.00	584.26	424.78
12	Prigoria	2,698.68	340.00	1,212.00	513.00	320.00	313.68
13	Berleşti	2,690.60	74.20	995.40	1,184.00	343.00	94.00
14	Licurici	2,579.78	351.70	665.19	1,086.04	343.39	133.46
15	Roşia de Amaradia	2,391.70	974.40	312.10	270.50	520.40	314.30
16	Aninoasa	2,385.82	284.30	242.60	950.30	530.40	378.22
17	Stăneşti	2,353.38	381.50	410.00	655.00	475.00	431.88
18	Dănciuleşti	2,336.00	250.00	505.00	453.00	998.00	130.00
19	Bumbeşti-Jiu	2,313.23	396.00	483.00	785.00	280.25	368.98
20	Logreşti	2,297.60	529.70	549.60	443.20	630.10	145.00
21	Muşeteşti	2,296.55	880.53	519.05	530.60	270.37	96.00
22	Alimpeşti	2,290.90	23.30	388.90	825.30	521.00	532.40
23	Godineşti	2,256.60	340.00	450.00	865.10	475.20	126.30
24	Bengeşti-Ciocadia	2,220.67	163.70	179.50	1,638.47	239.00	-
25	Motru	2,194.00	413.00	328.00	651.30	481.70	320.00
26	Săcelu	2,139.80	437.07	586.23	670.00	330.20	116.30
27	Stejari	2,054.04	539.73	460.28	741.00	230.00	83.03
28	Mătăsari	2,035.00	461.00	86.00	135.00	780.00	573.00
29	Bumbeşti-Piţic	2,022.20	420.00	620.00	450.00	340.00	192.20
30	Târgu-Jiu	2,020.00	999.00	230.00	625.00	98.00	68.00
31	Vladimir	1,974.00	230.00	205.00	1,405.80	133.20	-
32	Polovragi	1,971.58	337.80	867.50	339.00	170.00	257.28
33	Slivileşti	1,928.15	678.00	265.50	266.50	385.00	333.15
34	Brăneşti	1,909.27	112.30	339.10	1,093.87	329.00	35.00
35	Târgu-Cărbuneşti	1,830.00	630.00	469.00	630.00	50.00	51.00
36	Schela	1,800.58	293.30	846.80	219.20	190.20	251.08
37	Văgiuleşti	1,778.71	329.63	457.34	313.71	389.00	289.03
38	Bolboşi	1,678.70	323.70	46.40	803.10	380.20	125.30
39	Scoarţa	1,650.00	686.00	275.00	350.00	280.00	59.00
40	Turburea	1,635.46	408.20	455.78	321.00	340.00	110.48
41	Căpreni	1,628.25	376.66	304.93	590.40	230.00	126.26
42	Negomir	1,526.00	254.00	337.00	275.00	540.00	120.00

0	1	2	3	4	5	6	7
43	Plopşoru	1,477.00	125.00	187.00	780.00	308.00	77.00
44	Ciuperceni	1,470.10	551.20	42.70	109.00	713.00	54.20
45	Stoina	1,351.70	134.87	216.22	610.00	270.00	120.61
46	Fărcăşeşti	1,315.00	328.00	230.00	741.00	16.00	-
47	Turceni	1,256.63	521.94	315.69	197.00	125.00	97.00
48	Hurezani	1,211.53	28.35	304.48	119.36	629.34	130.00
49	Drăgoteşti	1,177.50	87.50	107.50	200.00	523.00	259.50
50	Albeni	1,150.70	100.40	184.70	425.20	280.50	159.90
51	Băleşti	1,145.90	246.80	34.00	625.00	184.10	56.00
52	Leleşti	1,084.00	317.00	417.00	204.00	98.00	48.00
53	Dăneşti	1,047.70	469.00	385.80	115.00	57.60	20.30
54	Samarineşti	1,015.00	115.00	198.00	517.00	94.00	91.00
55	Ţânţăreni	956.10	373.90	287.20	167.00	98.00	30.00
56	Câlnic	859.00	161.50	168.90	320.00	128.00	80.60
57	Săuleşti	850.00	176.00	213.00	231.00	143.00	87.00
58	Ioneşti	834.00	74.00	342.00	240.00	148.00	30.00
59	Bălteni	742.00	250.00	253.00	178.00	61.00	-
60	Turcineşti	682.30	152.70	80.40	289.00	120.00	40.20
61	Jupâneşti	678.20	159.90	117.10	220.10	80.00	101.10
62	Arcani	603.30	45.00	197.60	241.00	83.00	36.70
63	Cătunele	591.00	68.30	86.15	190.25	221.30	25.00
64	Bărbăteşti	573.80	97.40	119.80	270.90	68.90	16.80
65	Drăguţeşti	520.00	110.00	75.00	220.00	92.00	23.00
66	Ţicleni	502.30	115.01	102.29	120.00	110.00	55.00
67	Teleşti	491.50	77.30	125.00	63.00	123.20	103.00
68	Urdari	489.08	150.30	107.90	87.40	75.40	68.08
69	Glogova	466.00	80.00	98.00	191.00	97.00	-
70	Rovinari	311.00	78.00	115.00	56.00	28.00	34.00
	TOTAL	**134,940.26**	**24,569.57**	**28,655.82**	**44,478.10**	**26,160.83**	**11,075.94**

Source: Original data

In Novaci the strong, very strong and excessive erosion is manifested on 2,524.60 ha, which represents 66% of the area affected by erosion. In Baia de Fier, strong erosion affects 48.72% of the total area affected by this phenomenon.

The least affected by surface erosion are ATU Glogova with 466 ha, but where also strong and very strong erosion occupies the largest area, namely 288 ha, which is 61.80%, but also ATU Rovinari with 311 ha, where strong and very strong and excessive erosion is manifested on an area of 118 ha, which represents 37.94% of the area affected by erosion.

Thus, out of the total area affected by surface erosion, which is of 34,940.26 ha, mild erosion is manifested on 18.20%, moderate erosion on 21.24%, strong erosion on 32.96%, very strong erosion on 19.39%, and excessive erosion on 8.21%.

Depth erosion, also called linear or torrential, occurs on land with higher slopes, in directions of concentration of the runoff, where the process of detachment and transport of mineral and organic material is much more intense compared to surface erosion (Popescu et al., 2024).

Depth erosion causes great damage to agriculture through its formations, rills (Figure 3), gullies (Figure 4) and ravines (Figure 5), which can remove large areas of land from the agricultural circuit, prevent the mechanized execution of works, can affect industrial objectives, human settlements, communication routes, etc.

Depth erosion affects 4,087.69 ha representing 1.68% of the agricultural area of Gorj County.



Figure 3. Depth erosion – Rill in the Novaci - Rînca area, Gorj county



Figure 4. Depth erosion – Gully in the Novaci - Rînca area, Gorj county



Figure 5. Depth erosion – Ravine in the Novaci - Rînca area, Gorj county

Table 2 shows depth erosion on ATU and depth erosion formations.

The ATUs most affected by deep erosion are: Bălăneşti with 187.79 ha, followed by Cruşeţ with 142.98 ha, Alimpeşti with 137.50 ha, Bumbeşti-Jiu with 136.08 ha and Muşeteşti with 132.22 ha.

Table 2. Depth erosion on ATUs and erosion formations, in Gorj county

Nr. crt.	Administrative-Territorial Unit	Affected area (ha)	Erosion formations at depth		
			Rills	Gullies	Ravines
0	1	2	3	4	5
1	Bălăneşti	187.79	7.18	5.89	174.72
2	Cruşet	142.98	7.78	5.20	130.00
3	Alimpeşti	137.50	6.80	5.70	125.00
4	Bumbeşti-Jiu	136.08	10.14	8.41	117.53
5	Muşeteşti	132.22	7.08	7.50	117.64
6	Licurici	126.31	18.47	15.36	92.48
7	Hurezani	126.28	12.00	4.00	110.28
8	Bustuchin	125.48	12.00	15.00	98.48
9	Prigoria	118.76	10.40	9.86	98.50
10	Borăscu	114.59	8.08	6.31	100.20
11	Tismana	103.26	11.57	11.09	80.60
12	Scoarţa	98.00	5.80	13.20	79.00
13	Roşia de Amaradia	93.82	10.18	10.44	73.20
14	Bumbeşti-Piţic	89.90	3.80	5.60	80.50
15	Bolboşi	85.56	5.40	4.36	75.80
16	Berleşti	81.30	3.90	3.20	74.20
17	Stejari	81.21	8.78	6.28	66.15
18	Baia de Fier	78.79	6.80	8.49	63.50
19	Runcu	77.15	11.06	11.39	54.70
20	Ciuperceni	76.80	6.30	11.40	59.10
21	Aninoasa	73.88	5.67	4.21	64.00
22	Teleşti	72.10	8.90	12.30	50.90
23	Godineşti	71.16	6.56	5.60	59.00
24	Padeş	69.95	9.06	10.29	50.60
25	Albeni	66.10	21.50	16.10	28.50
26	Motru	59.50	-	4.50	55.00
27	Urdari	58.33	7.44	4.89	46.00
28	Căpreni	58.14	5.66	4.18	48.30
29	Glogova	58.00	4.00	11.00	43.00
30	Bărbăteşti	55.20	10.80	7.30	37.10
31	Arcani	55.00	5.70	2.30	47.00
32	Săuleşti	53.10	3.80	11.30	38.00
33	Polovragi	50.92	9.14	9.58	32.20
34	Dăneşti	49.13	5.04	4.09	40.00
35	Bengeşti-Ciocadia	45.30	10.30	5.20	29.80
36	Ţicleni	42.19	6.18	4.41	31.60
37	Schela	42.10	9.34	7.76	25.00
38	Ioneşti	41.90	4.42	2.48	35.00
39	Novaci	40.79	8.12	8.87	23.80
40	Ţânţăreni	40.59	5.80	3.61	31.18
41	Peştişani	39.44	8.24	9.10	22.10
42	Logreşti	39.29	7.20	8.09	24.00
43	Slivileşti	38.40	-	4.20	34.20
44	Crasna	36.43	8.60	5.53	22.30
45	Negomir	34.30	27.00	3.70	3.60
46	Stăneşti	33.89	9.40	8.19	16.30
47	Bălteni	33.70	7.40	3.30	23.00
48	Stoina	31.92	7.11	4.17	20.64
49	Samarineşti	31.70	2.80	3.40	25.50
50	Brăneşti	29.32	3.00	7.80	18.52
51	Plopşoru	29.30	-	-	29.30
52	Săcelu	29.07	6.42	4.85	17.80
53	Văgiuleşti	28.34	-	-	28.34
54	Leleşti	28.00	4.00	3.00	21.00
55	Mătăsari	27.00	3.80	2.50	20.70
56	Drăgoteşti	25.80	3.80	4.30	17.70
57	Târgu-Jiu	25.00	-	-	25.00
58	Turceni	24.83	6.14	3.40	15.29
59	Cătunele	24.00	-	2.00	22.00
60	Câlnic	23.91	6.82	4.39	12.70
61	Turburea	22.49	6.34	4.53	11.62
62	Fărcăşeşti	21.00	-	-	21.00
63	Jupâneşti	20.50	4.14	3.36	13.00
64	Băleşti	19.20	4.80	2.30	12.10
65	Vladimir	17.20	-	-	17.20
66	Rovinari	15.00	-	-	15.00
67	Turcineşti	12.50	3.00	4.20	5.30
68	Dănciuleşti	12.00	-	-	12.00
69	Târgu-Cărbuneşti	11.00	-	-	11.00
70	Drăguţeşti	6.00	-	-	6.00
	TOTAL	**4,087.69**	**450.96**	**404.96**	**3,231.77**

Source: Original data

Of the total area affected by the deep erosion of the Bălăneşti territory, the area affected by ravines is 174.72 ha, which represents 93%.

As far as Cruşeţul is concerned, the area occupied by ravines is also the largest,

representing 90.92% of the area affected by depth erosion.
The situation is no different in the case of Alimpesti either, in the sense that the area occupied by ravines represents 90.90% of the total area affected by deep erosion. From this it can be concluded that in these 3 ATUs, deep erosion, represented by ravines, affects these territories approximately entirely.
The ATUs with the smallest area affected by deep erosion are Drăguțești (6 ha), Târgu-Cărbunești (11 ha) and Dănciulești (12 ha), but in these ATUs, the entire affected area is occupied by ravines. These formations, which represent the most advanced stage of depth erosion and constitute a major danger to agriculture and the environment, fully cover (100%) the areas exposed to this process.

CONCLUSIONS

Soil erosion is the most extensive and most serious form of soil degradation in Gorj County, affecting both agricultural land and natural ecosystems. This phenomenon contributes to the loss of soil fertility, the reduction of arable areas and the increased risk of landslides.
Surface erosion is a major problem in Gorj County, affecting a total area of 134,940.26 ha, representing 55.36% of the total agricultural area of the county, having different degrees of intensity. Thus, out of the total area affected by surface erosion, moderate erosion (21.24%), strong erosion (32.96%) and very strong erosion (19.39%) are the most common, accumulating over 73% of the total affected area.
The ATUs most affected by surface erosion are Padeș, Crasna, Tismana and Novaci, where strong, very strong and excessive erosion occupies significant percentages of the total eroded area.
Surface erosion is influenced by the predominantly mountainous and hilly terrain, by the rainfall that is frequent in this region, as well as by human activities, all of which favour soil degradation processes.
Depth erosion (linear or torrential) affects 4,087.69 ha, which represents 1.68% of the county's agricultural area, having a negative impact on agriculture, infrastructure and the environment.
Bălănești, Crușeț and Alimpești are the most affected by depth erosion, ravines covering over 90% of the area affected by this phenomenon. The ATUs with the smallest area affected by erosion are Drăguțești, Târgu-Cărbunești and Dănciulești, however, in these areas, erosion is predominantly in an advanced form, through ravines, which represents a significant risk.
Soil erosion has a very high impact from an economic, ecological and social point of view, by reducing agricultural production and farmers' incomes, degrading biodiversity and natural habitats, as well as affecting local communities, exposed to the risks of landslides and forced migration due to economic difficulties.
In order to prevent and combat soil erosion, it is essential to implement measures such as afforestation of vulnerable areas, establishing the structure of crops according to the degree of erosion and the protection it provides to the soil, using a cropping system that ensures the reduction of the speed, flow and volume of water flowing on the slopes, shaping the land surface in order to retain as much precipitation as possible, the execution of special hydrotechnical works for the interception and evacuation of water from the slopes (earth embankments, terraces, coastal canals), as well as the adoption of conservation agriculture practices (minimum tillage or no-till, permanent soil cover and crop rotation). These actions contribute to reducing soil degradation and maintaining soil fertility in the long term.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

THE INFLUENCE OF SOIL TILLAGE AND FERTILIZATION ON SOME QUALITY PARAMETERS OF GRAIN SORGHUM PRODUCTION UNDER THE CLIATIC CONDITIONS OF THE SĂRĂȚENI- IALOMIȚA

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Abstract

Sorghum is a drought-tolerant crop that thrives in arid environments, which has earned it a reputation worldwide for its ability to grow in areas with limited rainfall, low soil fertility, and harsh conditions. Pedoclimatic conditions, along with the implementation of proper cultivation technologies, can increase thousand kernel weight (TKW) and protein content, ensuring better crop valorization. This research presents the results of a three-year study (2020-2023) aiming to identify the best interactions between different primary tillage practices and fertilization strategies based on nitrogen, phosphorus, and foliar fertilizers. The goal was to determine the optimal approach for non-irrigated sorghum crops in the Sărățeni region (Ialomița County) to enhance both protein content and TKW. Regarding TKW, most fertilized variants showed significantly higher values than the unfertilized control, with an increase of +6.4 g observed in the $N_{100}P_{50}$ + Borocal 1.5 L/ha (foliar) treatment. Nitrogen fertilization at 100 kg/ha significantly increased protein content to 9.3%, with a minimum gain of +0.8%. Soil tillage by subsoiling at 35 cm resulted in the highest protein content of 9.1%.

Key words*: Sorghum bicolor L., tillage, fertilization, protein, thousand kernel weight, hectoliter weight.*

INTRODUCTION

Sorghum is an adaptable and versatile cereal crop of significant importance in global agriculture (FAO). The remarkable resilience of *Sorghum bicolor* and its ability to thrive under diverse environmental conditions have earned it a reputation as a staple crop in many regions worldwide. Sorghum, a widely cultivated cereal belonging to the Poaceae family (Erbetta E., 2024), is known for its resistance and adaptability to various environmental factors, making it a key crop in many parts of the world (Abdel-Ghany S.E., 2020).

Sorghum protein is of interest due to its unique composition, which can contribute to healthy and sustainable diets. Firstly, sorghum is an important source of plant-based protein. Its protein content ranges from 8% to 14%, depending on the variety and growing conditions (Majercik et al., 2012). Compared to other cereals, sorghum has a favorable amino acid profile, being richer in essential amino acids such as lysine, which makes it an attractive option for vegetarian and vegan diets (Shabani et al., 2021). Sorghum protein is especially relevant in the context of growing interest in alternatives to animal-based proteins, aiming to reduce environmental impact (Yalçın et al., 2019). Another key aspect is the bioavailability of sorghum proteins.

Thousand kernel weight (TKW) is a significant factor in optimizing sorghum cultivation, with major implications for both yield and soil resource management. As a crop, sorghum stands out for its adaptability to diverse soil and climate conditions, including arid environments, a trait that becomes increasingly valuable under current climate change scenarios.

A study by Ailincăi et al. emphasized that proper fertilization and crop rotation management on Moldavian Plateau soils can improve both soil quality and sorghum yields. This highlights the importance of nutrient balance in enhancing agricultural productivity (Ailincăi et al., 2011). The research suggests that correct nutrient application is crucial to maximizing yield, which in turn can positively influence TKW at harvest.

MATERIALS AND METHODS

The research was conducted in the locality of Sărățeni, Ialomița County (44°38′11″N

26°55′41″E), between 2020 and 2023, on a cambic chernozem soil, within the "Bănică Ion" Individual Enterprise. The year 2021 recorded a rainfall regime above the average for the period, totaling 673.6 mm, which favored high production yields. Both during sowing and tillering stages, the amount of precipitation exceeded normal levels. During the vegetative development period (May-August) in 2021, 283.4 mm of rainfall were recorded, compared to only 96 mm in 2022 a difference of 184 mm. The year 2023 had an average rainfall regime, totaling 177 mm. The average monthly temperature during the vegetative period was 20.4°C, while the annual average for the 2020-2023 interval was 21.5°C, aligning with global warming trends.

RESULTS AND DISCUSSIONS

The Influence of Primary Tillage on Thousand Kernel Weight (TKW)

The data obtained from the experiment highlight a significant influence of primary tillage type on the thousand kernel weight (TKW) in sorghum cultivation (Table 1).

The control variant (plowing at 25 cm) recorded a TKW of 33.2 g, used as the reference value (100%). Subsoiling at 45 cm increased the kernel weight to 33.9 g, representing a gain of +0.7 g, statistically significant at the 0.1% threshold (***), suggesting improved plant development conditions under this treatment.

Table 1. The influence of primary tillage on thousand kernel weight (TKW)

Variant	TKW		Difference (g)	Significance
	g	%		
Plowing at 25 cm (control)	33.2	100	CONTROL	-
Subsoling at 45 cm	33.9	102.2	0.7	***
Subsoling at 35 cm	35.4	106.5	2.2	***
Disking at 10 cm	33.0	99.5	-0.2	
LSD 5% = 0.4 g				
LSD 1% = 0.6 g				
LSD 0.1% = 0.7 g				

The most pronounced effect was observed in the subsoiling at 35 cm variant, where TKW reached 35.4 g - a difference of +2.2 g above the control, also significant at the 0.1% level. This variant most efficiently promoted kernel formation and filling, possibly due to an optimal combination of improved soil aeration, water availability, and nutrient uptake.

On the other hand, shallow tillage through disking at 10 cm caused a slight decrease in TKW to 33.0 g, slightly below the control value, but the difference of 0.2 g was not statistically significant.

The results indicate that deeper tillage, especially subsoiling at 35 cm, positively contributes to grain mass development in sorghum, with direct implications on yield and production quality. This tillage method can be considered a valuable technological option under current pedoclimatic conditions in modern agriculture.

The Influence of Fertilization on Thousand Kernel Weight (TKW)

The control variant (N_0P_0) recorded a TKW of 31.4 g, serving as the baseline (100%). Applying nitrogen at a rate of 100 kg/ha ($N_{100}P_0$) increased kernel weight to 33.5 g, a gain of +2.1 g, statistically significant at the 0.1% threshold (***). This increase clearly indicates the effect of nitrogen on plant vigor and grain filling (Table 2).

The addition of phosphorus ($N_{100}P_{50}$) led to a TKW of 34.9 g, which is +3.5 g above the control, demonstrating the synergistic effect of the two major nutrients. This treatment had a strong positive impact on grain development, confirming the importance of nutritional balance in sorghum crop technology.

Table 2. The influence of fertilization on thousand kernel weight (TKW)

Variant	TKW		Difference (g)	Significance
	g	%		
N_0P_0	31.4	100	CONTROL	-
$N_{100}P_0$	33.5	106.7	2.1	***
$N_{100}P_{50}$	34.9	111.0	3.5	***
$N_{100}P_{50}$ + Borocal 1.5 l/ha (foliar)	37.8	120.4	6.4	***
Borocal 1.5 l/ha (foliar only)	31.9	101.4	0.5	*
LSD 5% = 0.5 g				
LSD 1% = 0.6 g				
LSD 0.1% = 0.8 g				

The highest TKW value was obtained in the $N_{100}P_{50}$ + foliar Borocal treatment, with 37.8 g -

a +6.4 g difference compared to the control, significant at the 0.1% level. This highlights the maximum efficiency of combining base fertilization with micronutrient supplementation, particularly boron and calcium, which support pericarp formation and sugar transport to the grains.
Foliar fertilization alone had a marginal effect, with a TKW of 31.9 g, a modest increase of +0.5 g, significant only at the 5% level (*). This suggests that in the absence of adequate base fertilization, foliar application alone cannot compensate for essential nutrient deficiencies.

The Influence of Tillage on Hectoliter Weight (HW)

Hectoliter weight (HW) is a key indicator of the physical and commercial quality of sorghum grains, reflecting their density and degree of filling. The results show a clear influence of soil tillage on this parameter (Table 3).
In the control variant, with plowing at 25 cm, HW was 61.8 kg/hl, used as the reference value (100%). Deeper tillage by subsoiling at 45 cm and 35 cm increased HW to 63.1 kg/hl and 63.4 kg/hl, respectively - differences of +1.4 and +1.6 kg/hl compared to the control, both statistically significant at the 0.1% level (***). These increases suggest a positive effect of deep soil loosening on the formation of dense, well-developed grains, likely through better water and nutrient supply during grain filling.

Table 3. The influence of primary tillage on hectoliter weight (HW)

Variant	HW		Difference (kg/hl)	Significance
	kg/hl	%		
Plowing at 25 cm (control)	61.8	100	CONTROL	-
Subsoling at 45 cm	63.1	102.2	1.4	***
Subsoling at 35 cm	63.4	102.6	1.6	***
Disking at 10 cm	61.0	98.8	-0.8	***
LSD 5% = 1.9 kg/hl				
LSD 1% = 2.5 kg/hl				
LSD 0.1% = 3.3 kg/hl				

In contrast, the disking variant (shallow tillage) yielded a lower HW value of 61.0 kg/hl, with a negative difference of -0.8 kg/hl compared to the control. Although the absolute value of the difference is small, it was statistically significant at the 0.1% level (***), reflecting a real negative impact on the physical quality of the grains.
These findings confirm that deep tillage, particularly subsoiling, improves the physical quality of production by increasing hectoliter weight - a key advantage for both processing and commercial valorization. In contrast, shallow tillage may limit the plant's physiological potential to produce well-filled and dense grains.

The Influence of Fertilization on Hectoliter Weight (HW)

The data presented in Table 4 reveal a significant influence of fertilization on test weight (TW) in 2021. The unfertilized control variant (N_0P_0) recorded a value of 60.1 kg/hl, serving as the baseline for comparison with the other treatments.
The application of nitrogen at a rate of 100 kg/ha without phosphorus ($N_{100}P_0$) led to an increase in test weight to 62.0 kg/hl. The 2.0 kg/hl increase was statistically significant at the 0.1% level (***). This suggests a positive effect of nitrogen even in the absence of phosphorus, likely due to the stimulation of metabolic processes involved in the formation of storage compounds.

Table 4. The influence of fertilization on hectoliter weight

Variant	HW		Difference (kg/hl)	Significance
	kg/hl	%		
N_0P_0	60.1	100	CONTROL	-
$N_{100}P_0$	62.0	103.2	2.0	***
$N_{100}P_{50}$	63.5	105.7	3.4	***
$N_{100}P_{50}$ + Borocal 1.5 l/ha (foliar)	65.1	108.3	5.0	***
Borocal 1.5 l/ha (foliar only)	60.9	101.4	0.8	
LSD 5% = 2.1 kg/hl				
LSD 1% = 2.8 kg/hl				
LSD 0.1% = 3.7 kg/hl				

The highest TW value was obtained in the $N_{100}P_{50}$ + Borocal treatment - 65.1 kg/hl. The increase of 5.0 kg/hl compared to the control exceeded the 0.1% significance threshold, confirming the effectiveness of supplementing base fertilization with a foliar Borocal treatment. This result suggests that the interaction between macronutrients and the micronutrients in Borocal plays an important role in improving crop quality parameters.

Also, the $N_{100}P_{50}$ variant (without foliar treatment) achieved an intermediate test weight value of 63.5 kg/hl significantly higher than the control (+3.4 kg/hl), but lower than the variant with Borocal highlighting the additional benefit of foliar application.
The variant treated solely with Borocal, without base fertilization, generated a test weight of 60.9 kg/hl, only 0.8 kg/hl above the control. This difference was not statistically significant, indicating that Borocal alone is insufficient to significantly improve test weight and acts effectively only in combination with adequate base fertilization.

The Influence of primary tillage on protein content

The results in Table 5 highlight the distinct effects of primary tillage type on protein content in sorghum grains under the 2021 conditions. Conventional plowing to 25 cm served as the control variant, with an average protein content of 8.8%, used as the reference point for comparison.
Soil tillage through subsoiling to 45 cm resulted in the same protein content as the control (8.8%), with no statistically significant difference. This suggests that, under the given pedoclimatic conditions, the additional tillage depth did not significantly influence nutrient availability.

Table 5. Influence of primary tillage on protein content

Variant	Protein		Difference	Significance
	Content (%)	%		
Plowing at 25 cm	8.8	100	CONTROL	-
Subsoling at 45 cm	8.8	100.2	0.0	
Subsoling at 35 cm	9.1	102.9	0.3	***
Disking at 10 cm	8.6	98.0	-0.2	ooo
LSD 5% = 0.2%				
LSD 1% = 0.2%				
LSD 0.1% = 0.3%				

In contrast, subsoiling at 35 cm led to a statistically significant increase in protein content, reaching 9.1%. The +0.3 percentage point difference compared to the control was significant at the 0.1% level (***), indicating that this working depth favored better root development and more efficient nitrogen uptake an essential nutrient for protein synthesis.
Minimum tillage, represented by disking to 10 cm, negatively affected protein content, lowering the average to 8.6%. The –0.2 percentage point difference from the control was statistically significant at the 1% level (ooo), confirming that shallow tillage limits root system development and access to nutrients in deeper soil layers.

The Effect of Fertilization on Grain Protein Content

Sorghum grain protein content responded significantly to the fertilization regime, confirming the importance of mineral nutrition especially nitrogen in stimulating plant protein metabolism.
In the unfertilized control variant (N_0P_0), protein content was 8.5%, serving as the reference value (100%). The application of nitrogen alone ($N_{100}P_0$) resulted in the highest protein content of 9.5%, an increase of +1.0% over the control - a statistically significant difference at the 0.1% level (***), confirming nitrogen's crucial role in protein biosynthesis (Table 6).

Table 6. Influence of fertilization on protein content

Variant	Protein		Difference	Significance
	Content (%)	%		
N_0P_0	8.5	100	CONTROL	-
$N_{100}P_0$	9.5	111.5	1.0	***
$N_{100}P_{50}$	9.3	108.8	0.8	***
$N_{100}P_{50}$ + Borocal 1.5 l/ha (foliar)	8.7	102.1	0.2	*
Borocal 1.5 l/ha (foliar)	8.3	97.9	-0.2	ooo
LSD 5% = 0.2 %				
LSD 1% = 0.3%				
LSD 0.1% = 0.3%				

Although the $N_{100}P_{50}$ treatment maintained a high protein value (9.3%), it showed a slight decrease compared to the nitrogen-only variant. While the difference was still statistically significant (*), the addition of phosphorus did not further enhance protein synthesis. It is possible that phosphorus had a more pronounced effect on other yield components, such as grain weight, at the expense of protein concentration.

Supplementing the $N_{100}P_{50}$ treatment with Borocal (1.5 l/ha) resulted in a decrease in protein content to 8.7%, a value close to the control but still statistically significant at the 5% level (*). This suggests a possible dilution effect, either due to increased biomass production or a specific physiological influence of Borocal's micronutrients (boron and calcium) on the carbon-to-nitrogen ratio.

The treatment with Borocal alone, without base fertilization, resulted in the lowest protein content—8.3%, a decrease of –0.2% compared to the control, statistically significant at the 0.1% level (ooo). This confirms that micronutrients, in the absence of nitrogen input, are insufficient to sustain effective protein synthesis.

The data clearly show that nitrogen is the key factor in boosting protein content. Its application, alone or in combination with phosphorus, leads to significant increases. On the other hand, foliar micronutrient applications cannot replace basic fertilization and may even reduce protein concentration under certain conditions.

CONCLUSIONS

The thousand kernel weight was significantly influenced by both soil tillage depth and the applied fertilization regime. The highest values were recorded when deep scarification was combined with complete fertilization ($N_{100}P_{50}$), emphasizing the synergistic effect of these agrotechnical practices on enhancing the physical quality of sorghum seeds. Moreover, the integrated application of nitrogen, phosphorus, and foliar treatment with Borocal resulted in the greatest hectoliter weight, suggesting a synergistic interaction that positively affected grain quality. Overall, the findings highlight a cumulative beneficial effect of soil tillage and fertilization on grain protein content. Deep tillage, particularly scarification, in conjunction with nitrogen fertilization, produced the most favorable results, underscoring the critical role of incorporating these technological factors into sorghum cultivation strategies to optimize grain quality.

Subsoling at a depth of 35 cm was the most effective in increasing grain protein content, whereas shallow tillage practices such as disking exerted a detrimental influence on this qualitative parameter.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

SOIL PHYSICAL PROPERTIES, SPECTRAL RESPONSE AND YIELD OF THE SUNFLOWER UNDER DIFFERENT TILLAGE SYSTEMS AT EZARENI FARM, IASI COUNTY, ROMANIA

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Abstract

The study analyzes the short-term effect of tillage systems on soil physical properties, spectral response and sunflower yield, was based on three tillage system NT (No-tillage); MT (Minimum tillage); and CT (Conventional tillage). Data collected include soil physical properties (PR: Penetration resistance, VWC-Soil water content, Ks-Soil hydraulic conductivity), crop's spectral response through different multispectral sensors, and yield. Results showed that the for Ks, the highest value was obtained with NT at depth 0-10 cm. Concerning PR, high values were respectively recorded with CT (3.54 Mpa) during the vegetative period of the plant and with MT (2.54 MPa) during the harvesting period. Grain yields were also influenced by the tillage systems and reached values of 2.105, 2.631 and 3.052 kg/ha, respectively for the NT, MT and CT systems. Yield was also affected by heavy draught.

Key words*: tillage systems, soil physical properties, spectral response, yield, sunflower.*

INTRODUCTION

Soil is an essential resource for biodiversity, soil fertility, and food security on a global scale. Its maintenance and sustainable management are therefore crucial in the face of the challenges posed by population growth and environmental change. Among the most effective strategies for preserving soil health are crop rotation, reduced chemical use, and tillage systems. These practices not only prevent phenomen such as erosion and biodiversity loss but also restore the ecological and agricultural functions of soils, ensuring their ability to support future food and environmental needs.

Tillage represents a fundamental agronomic practice that has been integral to the majority of agricultural systems for centuries (Busari et al., 2015). Seedbed development, soil aeration, reducing soil evaporation, increasing plant water availability, breaking soil crusts, controlling pests, and incorporating residues are some of its aims (Amézketa, 1999; Köller, 2003). Additionally, tillage practices seek to decrease nutrient loss while enhancing plant root penetration and soil nutrient redistribution for effective nutrient absorption (Wang et al., 2015). Conventional tillage (CT) was an important factor in the development of modern agriculture practices since it helped to increase crop yields and manage weeds (Ashapure et al., 2019). In essence, plowing completely inverts the soil, which is what defines the CT. On the other hand, no-tillage (NT) and non-inversion tillage methods are part of conservation systems (minimal tillage, MT). With these approaches, crops are planted with reduced to no soil disturbance and maintaining a minimum of 30% mulch cover to protect the soil surface (Giller et al., 2015).

Similar to many regions in Europe, the adoption of conservation tillage in Romania remains restricted, however interest is gradually increasing (Țopa et al., 2021). Research on agricultural tillage systems in Romania and worldwide has focused on enhancing soil structure, reducing soil compaction, improving hydrological and aeration conditions, increasing organic matter content, optimizing the activity of microorganisms, understanding the effects of chemical substances, and identifying critical pollution thresholds (Raus et al., 2016)

Compared to conventional tillage (CT), minimum tillage (MT) and no-tillage (NT) systems have become widespread adoption due to their potential to reduce fossil fuel

consumption, enhance soil carbon sequestration, improve soil structure, and improve water infiltration. All of which contribute to maximize farm sustainability and production (Deubel et al., 2011; Ciri´c et al., 2012; Villamil and Nafziger, 2015; Zhao et al., 2017). The lack of soil plowing may worsen topsoil compaction, particularly in clayey soils and/or soils with low organic matter concentration (Secco et al., 2009; Franchini et al., 2012). Conversely, CT systems are recognized to typically diminish soil bulk density (BD) in the tilled layer (Dam et al., 2005; Wang et al., 2015) while enhancing soil porosity, hydraulic conductivity, and infiltration rate in the short term (Lampurlane and Cantero-Martínez, 2006; Schwen et al., 2011; Maharjan et al., 2018). Moreover, quantitative evaluations of crops under conventional tillage (CT), minimum tillage (MT), and no-till (NT) sometimes indicated contradictory, yielding varying responses conditional with the crop species (Salem et al., 2015; Pittelkow et al., 2015).

Plants potential to establish wide root systems is related to their capacity to gain nutrients and water from the soil (Chen and Weil, 2011; Dias et al., 2015). Tillage practices have the potential to promote soil compaction because they progressively change the physical characteristics of the soil. This result could limit plant access to subsurface water and root development during the growing season. This impact may, therefore, restrict plant development and alter its spectral response (Martins R.N. et al., 2021). Vegetation indices (VI) may be used to monitor the spectral characteristics of crops. The most widely and reliable VI is the normalized difference vegetation index (NDVI), which is derived from the reflectance of red and near-infrared (NIR) wavelengths (Rouse et al., 1973).

According to the research, there is a strong correlation between the NDVI and various biophysical factors, including agricultural production and biomass (Kross et al., 2015; Geipel et al., 2016; Zhou et al., 2017; Martins R.N. et al., 2021). In addition, over the years and with so many research studies carried out with a view to obtaining NDVI, a range of methods, remote sensing techniques and different sensors have been used. As an example, the space platforms used by Sakamoto et al. 2013, the aerial platform method used by Neiffield et al., 2015; Gabriel et al., 2017, uncrewed aerial vehicles (UAV) and proximal sensors (Stamatiadis et al., 2010; Hassan et al., 2019) were used to obtain NDVI.

With the emergence of drones, their use has become more frequent, given their flexibility, ease of handling, high spatial resolution, and ability to collect data on demand. As a result, drone systems enable accurate, high-resolution analysis and monitoring of crop time series quickly, non-deformably, and without extensive fieldwork (Yeom et al., 2019). Since tillage systems have a direct influence on the physical, chemical, and biological properties of the soil, this might also influence plant growth and development, and consequently their spectral response. However, the spatial resolution of UAV imagery is much finer and more exact than that of traditional remote sensing platforms, making it possible to observe variations in the growth-development process of plants and in their spectral response (Martins et al., 2021). The general objective of this study was the assessment of the short-term influence of three tillage systems on soil physical properties, spectral response, and sunflower crop yield.

MATERIALS AND METHODS

Study area

The study was conducted at the Ezăreni Research and Student Practice Station in Iasi, Romania (47°12′ N latitude, 27°51′ E longitude). The research was carried out on a soil type, characterized as Chernozem (WRB Classification) and clay-loam in texture with 36.1% clay, 26% silt and 37.9% sand. Its humus content varies between 2.7 and 3.4% and has a slightly acidic pH, according to the Romanian Soil Taxonomy System (SRTS 2012). The experimental research was carried out from April 2024 to October 2024 on the sunflower crop (*Helianthus annuus* L.) (Figure 1). This field area has an annual mean temperature 12.40°C and precipitation of 53.2 mm in 2024. (Figure 2), presents a 5% inclination and is located 126 m from sea level.

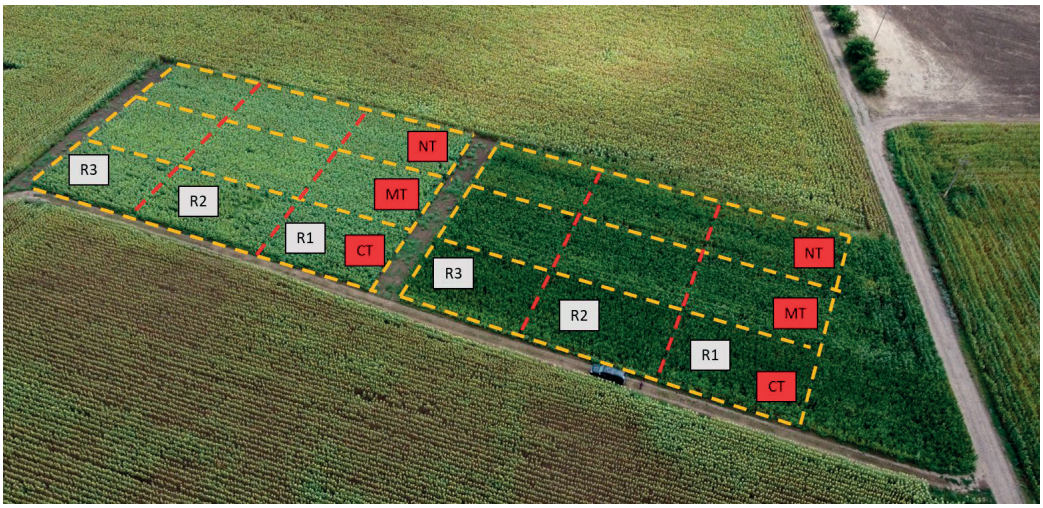


Figure 1. Experimental design on July 26th, 2024
(CT = Conventional tillage; MT = Minim tillage; NT = No tillage; R = Repetition)

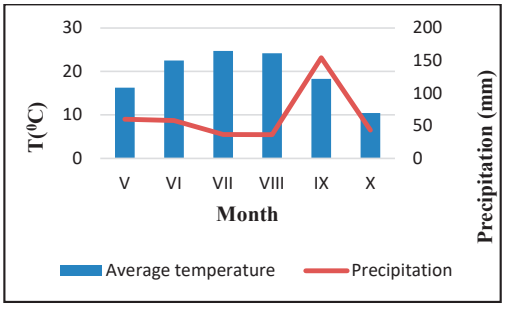


Figure 2. Precipitation amounts and mean monthly temperatures for April-October 2024

Experimental design and analysis of soil physical properties

Three treatments were used in this study, which was set up in a randomized block design: NT means direct seeding, MT with a chisel plow, and CT, whose soil was prepared with plow and two disk harrow passes at a depth of 0 to 30 cm and one plow pass. The sunflower hybrid HTS SUMIKO, from Syngenta was sown on April 30, 2024, in all three treatments at the time.

Soil penetration resistance (PR), hydraulic conductivity (Ks) were assessed in each tillage system in three different periods: sowing, vegetative period and harvest. For the PR, measurements were carried out with Eijkelkamp Penetrologger. It can measure depths of up to 80 cm and is equipped with a GPS system to quickly locate the measuring point. Measurements are taken directly in the field. Before the measurements start, a plan of the entire experimental field is drawn up, including all the variants of the experiment. Field measurements will begin once a working plan has been created using the penetrometer software. One of the parameters that should not be neglected in the measurements is the speed at which the penetrometer tip penetrates the soil, with the manufacturer recommending a speed of 2 cm/s. Ks was measured using a falling-head permeameter (Hauben water permeameter, Eijkelkamp Agrisearch Equipment) under conditions of instationary flow. For the Ks measurements, the samples were pre-saturated and then flooded for the measurement. The Ks was calculated by Equation (1) (Lal R. et al., 2004, Eijkelkamp, 2017):

$$k = \frac{a \times 1}{A \times t} \times ln \frac{h1}{h2}, \quad (1)$$

where: a is the burette area (cm^2); l is the height of the stainless-steel cylinders (cm); A is the area of the stainless-steel cylinders (cm^2); t is the time interval (s); h1 and h2 are the water level at the start and end points of the measurements (cm). Spectrum Technologies Water Scout SM100 (Spectrum Technologies, Aurora, IL) were installed on May 31, 2024 at 10, 20 and 30 cm soil depths to monitor soil moisture. At a depth of 30 cm, SM300 sensors were used to measure, temperature and electrical conductivity. Soil moisture content was

recorded as a percent volumetric water content at 60 min intervals continuously throughout the study period by WatchDog 1000 micro weather field station (Figure 3). Data were downloaded monthly to a computer using Spectrum technologies SpecWare 9 pro software. The mean for every day was calculated from 60 min interval data and graphed for the whole study period. The manufacturer specifications provided by Spectrum Technologies for Waterscout SM100 sensor indicated that the sensor resolution for volumetric moisture measurement is 0.1% with an accuracy of ±3% at E_C <8 mS/cm and 0.5 - 80°C temperature range (Spectrum Technologies, 2019).



Figure 3. WatchDog 1000 Micro Weather Field Station with moisture sensor

The spectrum parameter extraction

Multispectral mapping of the experimental field was performed on 21 August 2024, using the DJI Mavic 3 Multispectral UAV. The obtained images were processed with Pix4D software, and vegetation index maps were created. The pixels inside the plot areas were averaged to calculate the VI. Lastly, Equation (2) was used to get the NDVI for both cameras (Rouse et al., 1973):

$$NDVI = (Rnir - Rred)/(Rnir + Rred) \quad (2)$$

where: Rnir, represents the near-infrared reflectance; Rred, the red reflectance.

To confirm whether the soil tillage systems under evaluation such as CT, MT, Or NT had an effect on plant growth and spectrum response, the NDVI was applied.

Assessment of grain production

At the physiological maturity stage (October 05, 2024), sunflower plants were harvested manually from an area of 10 m^2. This method was repeated three times for each system and repetition. Then, the following yield components number of head, number of achenes/head, diameter of head, 1000-Kernel Weight (1000-KW), Test Weight (TW), achene moisture content, and the grain yield (kg ha^{-1}) considering the moisture of 5.01%.

Statistical analysis

Analysis of variance (ANOVA), adapted to the randomized complete block design, was used to analyse the data obtained. Significant differences between treatments were calculated using the Tukey' test ($P<0.05$). Statistical analyses were carried out using SPSS 26. 0 for windows.

RESULTS AND DISCUSSIONS

Effects of tillage systems on soil physical properties

Soil hydraulic conductivity (Ks)

Table 1 indicates that the highest Ks values were recorded respectively under NT (1.90 x 10^{-2} cm/s) and MT (1.53 x 10^{-2} cm/s) at the same depth (0-10 cm) during the vegetative period. During the harvesting period, the highest values were still observed under the same systems, but this time at different depths (20-30 cm) for NT (1.83 x 10^{-2} cm/s) and (1.53 x 10^{-2} cm/s) associated at (10-20 cm) for MT. The lowest Ks values were recorded under the CT both during the vegetation stage and at harvest, at the same depth of (10-20 cm). A significant difference ($P<0.05$) exists between the different tillage systems studied.

Table 1. Effect of tillage on hydraulic conductivity (x 10^{-2} cm/s)

Tillage system	Depth (cm)	Ks sowing	Ks vegetation	Ks harvesting
NT	0-10	0.55 ±0.000 a	1.90 ±0.003 g	0.70 ± 0.001 c
	10-20	1.08 ±0.000 b	0.72 ±0.000 bc	1.45 ± 0.000 e
	20-30	1.33 ±0.000 c	1.57 ±0.001fg	1.83 ± 0.001 f
	30-40	1.71 ±0.000 d	0.35 ±0.001 ab	0.58 ± 0.006 bc
MT	0-10	0.55 ± 0.000 a	1.53 ±0.001 efg	0.66 ± 0.000 c
	10-20	1.18± 0.000 b	1.03±0.000 cd	1.53 ± 0.002 e
	20-30	1.33 ± 0.000 c	0.41 ±0.000 ab	1.08 ± 0.000 d
	30-40	1,71 ± 0,000 d	0.52±0.000 b	0.46 ± 0.000 abc
CT	0-10	0.55 ± 0.000 a	1.31 ±0.001 def	0.22 ± 0.000 a
	10-20	1.08 ± 0.000 b	0.10 ±0.000 a	0.24 ± 0.000 a
	20-30	1.33 ± 0.000 c	1.13±0.000 d e	0.32 ± 0.000 ab
	30-40	1.71 ± 0.000 d	0.42 ± 0.000ab	0.38 ± 0.000 ab
	P	0.000	0.000	0.000

a, b, c, d, e: Means for the same column with different letters are significantly different at the 5% threshold: P<*0.05*.

Volumetric soil water content (VWC)

The soil moisture progression trend in the experimental field was observed at three soil depths from May 31 to October 8, 2024 (Figure 4).

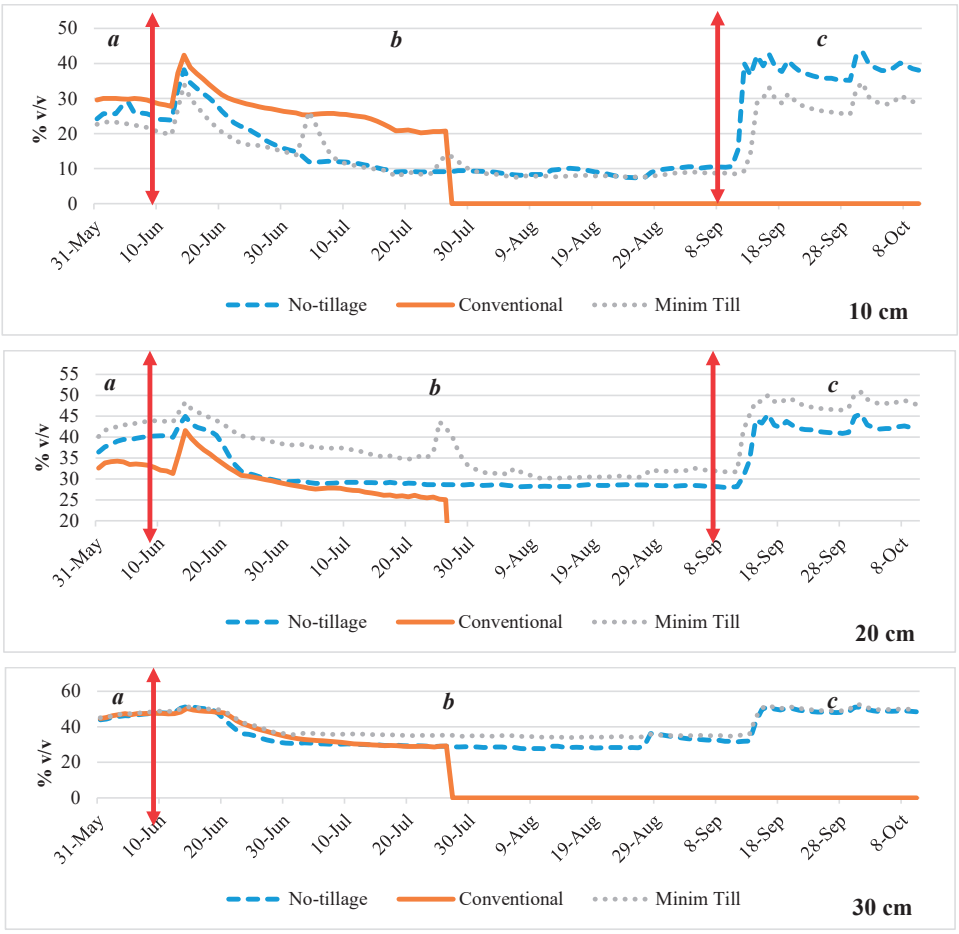


Figure 4. Effect of tillage systems on the volumetric soil moisture
(a = Sowing and emergence; b = Growing season; c = Maturity)

Soil electrical conductivity (Ec)

The SM300 sensors were installed in each tillage system at a depth of 30 cm to measure Ec. The results (Figure 5) show a significantly better Ec in the MT, with a maximum of 1.004 mS/cm reached on June 15, representing the beginning of the growth period, and a minimum of 0.325 mS/cm obtained on September 12, corresponding to the end of the growth period. On the other hand, the lowest EC values were recorded with NT. The lower Ec values recored under NT compared to CT and MT are could be due to differences in volumetric soil water content and evaporation rates. Endeed, NT maintains more stable soil water content levels, which conserves soil salinity, while CT increases evaporation resulting in decreased Ec. MT presents an intermediary environment that supports higher soil water content and root activity, which can justify the significant Ec values obtained especially in the vegetative stage.

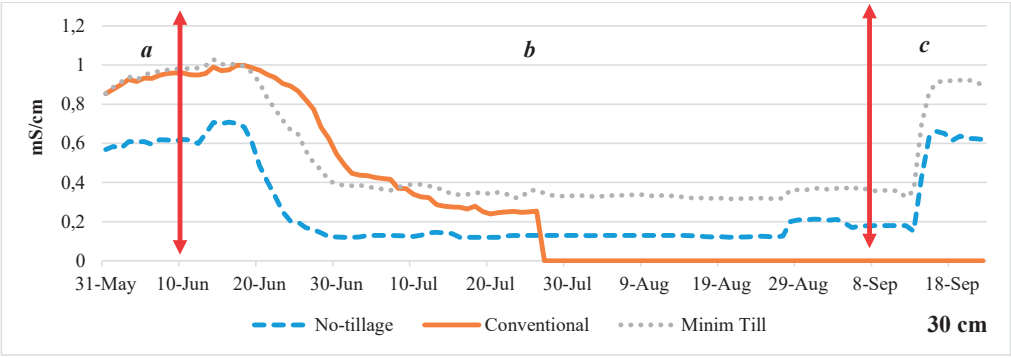


Figure 5. Effect of tillage systems on the soil electrical conductivity

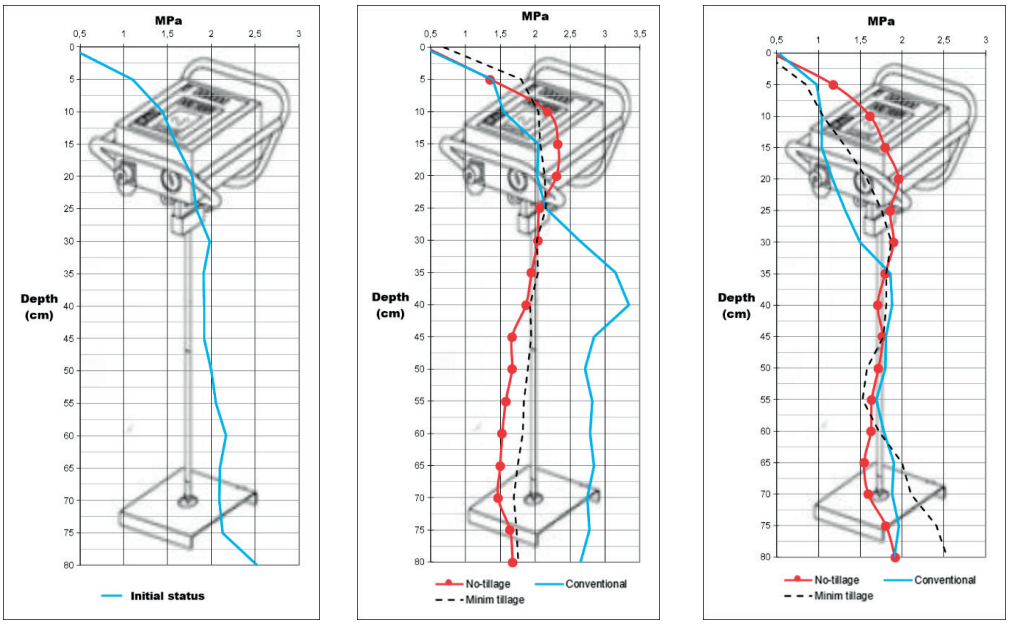
Penetration resistance (PR)

Results from the initial stage showed that PR values increased with soil depth, with minimum PR values observed at 0 to 5 cm and maximum values at a depth of 75 to 80 cm (Figure 6).

Based on results recorded during the vegetation stage of the sunflower crop over a depth interval of 0-80 cm (Figure 6).

Values in the NT range from a minimum of 0.42 MPa obtained at a surface depth of 0-5 cm to a maximum of 2.33 MPa obtained at a depth of 10-15 cm. In the MT, PR values oscillate between a minimum of 0.69 MPa and a maximum of 2.15 at depths of 0-5 cm and 20-25 cm, respectively. As for CT, the lowest PR value (1.39 MPa) is associated with the 0-5 cm depth, while the highest value (3.34 MPa) is associated with the 35-40 cm depth.

The results obtained during the harvesting stage showed that PR values did not increase as a function of soil depth, with the minimum PR values being observed at a depth of 0-5 cm and were (0.35 MPa) for MT; (0.45 MPa) for NT, and (0.52 MPa) for CT, respectively. The maximum values were obtained at different depths for each of the studied tillage systems. The maximum PR value in the NT (1.96 MPa) is associated with a depth of 15-20 cm. With MT, the maximum value (2.54 MPa) was observed at a depth of 75-80 cm. With CT, the maximum value (1.96 MPa) was recorded at depths of 70-75 cm (Figure 6).



Sowing *Vegetation stage* *Harvesting*

Figure 6. Effect of tillage systems on the penetration resistance

Influence of tillage systems on the spectral response of sunflower crop

The mean NDVI value indicates the mean state of health or vegetation cover in the area studied. NDVI values range from -1 to 1. Values between 0 and 1 indicate healthy, dense vegetation.

Values between 0 and -1 indicate sparse or affected vegetation.

Under the NT (Figure 7 a), the average NDVI value obtained is 0.536 and lies within the 0 to 1 interval, indicating average health and vegetation cover in the sunflower crop.

The mean NDVI value under the MT is 0.458 (Figure 7 b), which falls within the range of 0 and 1, but is lower than the mean NDVI of 0.536 observed under the NT.

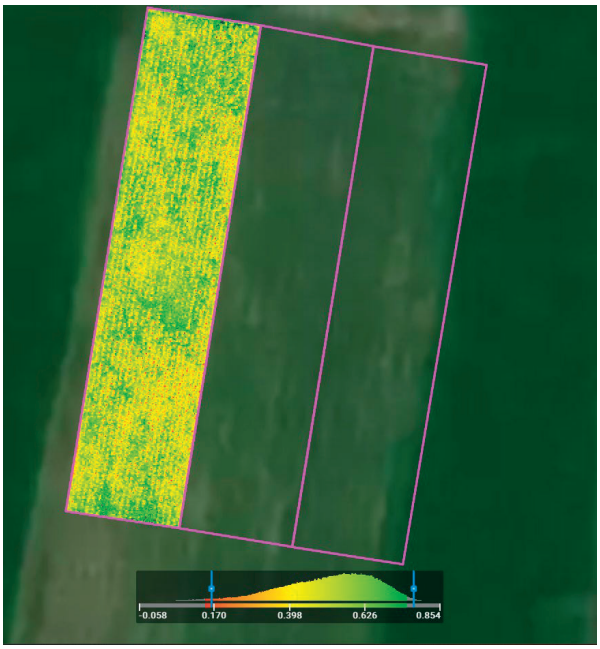
This shows that the sunflower crop under the MT has slightly lower overall vegetation health than under the NT.

NDVI values closer to 1 represent healthy vegetation, and this value (0.458) is below the tolerance threshold for moderate vegetation cover.

The mean NDVI value was 0.391 (Figure 7 c). This shows that the overall vegetation health of the sunflower crop under the CT is relatively poor.

An average NDVI of 0.391 is at the lower end of moderate vegetation health, indicating that the influence of tillage, modifying the physical properties of the soil, has also acted on the vegetation cover and/or vegetation of the sunflower crop.

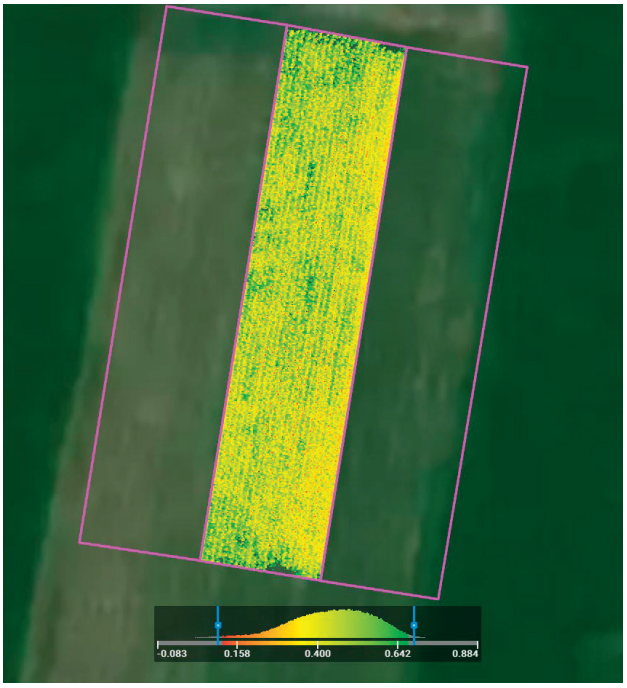
The cumulative results of the analyses show that the vegetation cover or condition of the sunflower vegetation under the CT is the lowest and, therefore, more affected than under the MT and NT.



Statistics

Layer area	999.846 m^2
Mean index	0.536
Index SD	0.136
Mean index (visible)	0.536
Index SD (visible)	0.128
Selected minimum value	0.141
Selected maximum value	0.752

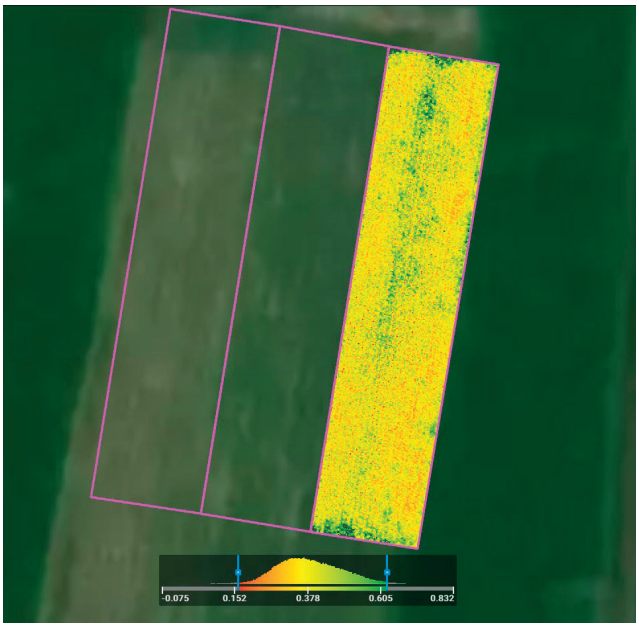
Figure 7 a. Effect of NT on the spectral response of sunflower crop



Statistics

Layer area	995.823 m^2
Mean index	0.458
Index SD	0.129
Mean index (visible)	0.452
Index SD (visible)	0.117
Selected minimum value	0.113
Selected maximum value	0.671

Figure 7 b. Effect of MT on the spectral response of sunflower crop



Statistics

Layer area	981.694 m^2
Mean index	0.391
Index SD	0.105
Mean index (visible)	0.389
Index SD (visible)	0.095
Selected minimum value	0.160
Selected maximum value	0.64

Figure 7 c. Effect of CT on the spectral response of sunflower crop

Effects of tillage systems on sunflower yield and yield components

The results obtained on yield parameters and sunflower yield show that, except for test weight (TW), tillage did not significantly influence the other parameters, which are 1000-Kernel weight (1000-KW) and WSH (weight of seed per head) (Table 2). The highest yield was obtained with CT. Between WSH and grain yield, there is a contrast whereby the weight of seed per head under NT is higher than that obtained under CT and inversely for yield. this contradiction can be explained by a higher plant density, which may lead to lower seeds per plant under NT, while the higher total yield in CT is due to a lower plant density but higher seeds, which contribute to a higher total yield.

Table 2. Effects of tillage systems on sunflower yield and yield components

Tillage system	WSH (g)	TW (kg/100 l)	1000-KW (g)	Yield (kg ha^{-1})
NT	113.59 ± 25.39a	41.82 ±0.15b	62.45 ±6.92a	2105
MT	95.82 ± 17.10a	40.51 ±0.34a	61.16 ±4.37a	2631
CT	101.91 ± 23.09a	43.08 ± 0.67c	55.85 ±7.19a	3052
P	0.621	0.000	0.446	-

a, b, c, d, e: Means for the same column with different letters are significantly different at the 5% threshold: $P<0.05$.

The influence of tillage systems on the physical properties of the soil, the spectral response and the yield of sunflowers were evaluated under the conditions of the Ezareni Research and Student Practice Station in Iasi. One of the properties studied was soil hydraulic conductivity (Ks). The results show that during the vegetation period and the harvesting phase, the highest value of Ks was obtained under NT (1.9 x 10^{-2} cm/s) and MT (1.5 x 10^{-2} cm/s) and at the same depth (0-10 cm). The difference in these results is that during the harvesting phase, the highest values were registered at a depth of 20-30 cm for the NT (1.8 x 10^{-2} cm/s), whereas with the MT (1.5 x 10^{-2} cm/s), they were recorded at 10-20 cm. The lowest Ks values were recorded under the CT both during the vegetation stage and at harvest at the same depth of (10-20 cm). A significant difference ($P<0.05$) exists between the different tillage systems studied. This highly low Ks observed under the CV is explained by the destruction of the earthworm community at ground level due to tillage. The presence of earthworms, combined with decomposing plant

roots, creates macropores. These macropores cannot be preserved in plowed soils. This considerably reduces water movement from the surface to the topsoils (Burtan et al., 2020). On the other hand, they are well preserved in no-till soils. Hence, the high Ks values obtained under NT and MT. These results are similar to those obtained by Osunbitan et al. (2005), Green et al. (2003), Alvarez et al. (2009) who affirm in their research that although the volume of macropores generally represents only a small fraction of the soil volume, its contribution to the hydraulic conductivity of the soil is very high.

Considering all the soil depths studied, the most significant increase in VWC was recorded during the second half of September, culminating between 18 and 28 September 2024. Not only was this the period in which the highest amount of rainfall was recorded (154 mm), but it is also correlated with the plants maturity stage. At a depth of 0-10 cm, the highest value was reached under NT compared with MT and CT. This result is in accordance with that obtained by Agbede (2010), Salem et al. (2015) and Semenikhina (2020), who found that the most significant VWC values were obtained under the NT. The results for depths of 10-20 cm and 20-30 cm show a higher value of VWC under the MT system. These results confirm those obtained by Ozgoz et al., (2007), who indicate in their studies that the VWC increases significantly under the MT system in their studies.

The Ec values obtained under NT are significantly lower than those recorded under CT and MT at a depth of 0-30 cm. This result is similar with that obtained by Lilienfein et al (2000). This shows that the Ec is lower under the NT and higher under the CT. This can be explained by the hypothesis that nutrient runoff is more intensified under NT than under CT. Significant Ec values were obtained on the MT, particularly during the vegetation phase starting at the beginning of July, when a decrease was observed under the CT, whereas under the NT the values remained constant during the same period.

Penetration resistance is a widely measured physical property of soil, as it represents a reliable method of assessing soil strength. Penetration resistance (PR) varies from one tillage system to another. Soil penetration resistance for the three tillage treatments increased with depth. Detailed analysis of each period of the plant cycle reveals some significant differences. Penetration resistance at a depth of 0 to 25 cm was higher under NT than under MT and CT. However, from a depth of 25 cm to 80 cm, resistance to penetration decreased, which coincides with its increase under the other two systems, MT and CT, where a highly significant difference was noted. On the one hand, this result confirms those of several authors who have stated in their studies that resistance to penetration is reduced in CT compared with NT (Sidiras et al., 2000; Taser and Metinoglu, 2005; Vakali et al., 2011; Afzalinia and Zabihi, 2014, Salem et al., 2015; Burtan et al., 2020; Martins et al., 2021) and on the other hand, these results are also in line with those obtained by, who have shown in their research that when resistance to penetration decreases in NT, it increases in the other systems (Osunbitan et al., 2005; Mihu et al., 2022).

The results of the NDVI spectral response analysis of sunflower showed a significant difference between the three systems studied (NT>MT>CT). These results contradict those obtained by Martin et al. (2021) in their study, in which they found no significant difference between the treatments evaluated (NT, MT, CT) in terms of spectral response and bean growth. According to the author, this may be associated with the low value obtained at PR not reaching the limit to affect root development. Leite et al. (2012) in their study, after evaluating four different types of tillage systems, the author observes a significant difference in the spectral response of NDVI on bean crops. The author claims that this difference is due to the high PR values obtained under NT and MT.

Similar high PR values were also recorded in our study under NT and MT, which justifies the significant difference noted between the treatments evaluated on the spectral response through NDVI of the sunflower crop.

In addition to PR, SWC also represents a factor influencing plant growth and vegetation and, contributing to the significant difference in results obtained.

The tillage system effect was significant on TW, under CT, where the highest values were reached. On the other hand, there was no

significant difference between 1000-Kernel weight (1000-KW) and WAC.

A significant difference was recorded in sunflower grain yield between the three systems studied, and the highest yield was obtained under CT. This result is confirmed by several authors who have shown that yield production is high under CT. However, the low yields obtained under all three tillage systems were due to the low rainfall recorded during the experiment.

CONCLUSIONS

A short study was carried out on the influence of three different soil systems on their physical properties and spectral response on sunflower production. The results obtained during this study indicated that Ks, PR and VWC performed most significantly and had the highest values under NT. However, a specific point can be observed at depths of 10-20 cm and 20-30 cm, where the highest values (50.74% v/v) and (52.25% v/v) were obtained under MT. Also, the PR values obtained (3.34 MPa) under CT (2.33 MPa) under NT, and, especially during the vegetative period, being above the threshold (2.5 MPa), could influence sunflower crop growth and yield. On the contrary, conventional tillage practices increased the Ec, and the highest values were recorded under this system. In terms of the spectral response of the crop through NDVI, there was a significant effect between the tillage systems studied. Regarding sunflower grain yield, a significant influence was observed between the three tillage systems evaluated, with a high yield under CT.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

RESEARCH ON THE INVENTORY OF THE MAIN PHYSICAL-HYDRIC AND CHEMICAL PROPERTIES OF THE MOLLIC PSAMOSOL IN THE SOUTHERN AREA OF OLTENIA (LEFT OF THE JIU RIVER)

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Abstract

The paper presents the main properties of the mollic psamosols from the left bank of the Jiu River, starting from the climatic conditions, the lithology of the area, hydrography, hydrology, natural vegetation. The research included the documentation, field and laboratory stages. This type of soil has somewhat better relations with water and air, is richer in the colloidal complex, in humus and nutrients, for this reason it has somewhat higher fertility compared to other sandy soils. Based on these properties and the natural conditions in the area, bonitation grades can be assigned and classes of favorability can be established for different crops. It should be taken into account that in the climatic conditions of recent years in Oltenia, in order to improve the mollic psamosols, a series of measures must be taken, including: deep loosening, enrichment in humus and clay, application of organic and chemical fertilizers, but especially the completion of the moisture deficit through irrigation.

Key words*: soil profile, horizon, physical-hydric and chemical properties, psamosol.*

INTRODUCTION

Of the total area of psamosols, the largest part is located in the western part of the Romanian Plain: Blahniței Plain, Băilești Plain and Caracalului Plain, being known as the sands of southern Oltenia. Therefore, psamosols (sands and sandy soils) occupy the largest part of southern Oltenia, starting from Ostrovul Corbului and extending to near Turnu Măgurele, over a length of 200 km and a width ranging between 3-30 km. The dune area itself occupies a strip with an average width of 10 km. The sands and sandy soils on the left side of the Jiu River appear as a strip whose width varies, and in the eastern part of the Leu-Rotunda Plain it is very sinuous.

The main task of agricultural specialists is to increase agricultural production in terms of quality and quantity. The achievement of this very important task in the current context of the food crisis depends, among other things, on the in-depth knowledge of the soil as the main means of production in agriculture (González-Moreno, 2016; Griesbach, 1998). Through pedological research, the soil profile with all its physical, hydrological and chemical properties can be known in detail. The evolution of these soil properties becomes important in the context of climate change, in the studied area, phenomena leading to desertification have been manifesting in recent years (International Organization for Standardization, 2010).

In order to know the types of soils found on the sandy area on the left bank of the Jiu River and their agro-productive potential, complex research has been carried out since 1955, starting with the Tâmbureşti sands. The results of research conducted at the resort over the years have made it possible to cultivate these sandy soils through modeling-leveling works, combating wind erosion, ameliorative fertilization and irrigation-drainage, as well as in the development of specific technologies for cultivating cereals, technical, fodder, medicinal and vegetable plants, fruit trees and vines.

The concept of sustainable agriculture, which is discussed in all countries with significant agricultural potential, requires an appropriate technology that takes into account the protection of natural resources, the maintenance or increase of the productive capacity of sandy soils, the use of constantly improved technologies, the saving of material and energy resources, the obtaining of high qualitative and quantitative productions. Pedological studies on sandy lands appear as an objective necessity in order to specify the methods of their rational exploitation, taking

into account the fact that their properties are modified by agricultural use, which makes it necessary to be constantly up to date with the situation regarding the properties and productive capacity of these lands (Blaga, 1994; Mihalache, 2014). The work aims to make a modest contribution to the knowledge of the agro-pedological properties of the sandy soils on the left bank of the Jiu River, the Craiova-Dăbuleni area, and to provide agricultural workers with data that will contribute to the intensive and rational exploitation of these lands.

MATERIALS AND METHODS

The research was carried out in the field and in the laboratory where the following aspects were sought to be pursued (Călina, 2000; 2017):

1 - knowledge of the natural conditions for the formation of psamosols on the left bank of the Jiu River, Craiova-Dăbuleni area. This aspect required an in-depth study from a geomorphological, lithological, hydrographic, hydrological, climate and vegetation point of view of the studied area;

2 - identification and study of the main psamosols on the left bank of the Jiu River, Craiova-Dăbuleni area. The documentation was done in the field by executing and studying soil profiles at points covering the entire studied area and documentation at the specialized institution OSPA Craiova;

3 - determination of the main physical, hydrophysical and chemical properties of the psamosols on the left bank of the Jiu River, Craiova-Dăbuleni area. Samples were taken from the profiles made in the field and analyzed in the laboratory;

4 - establishing the suitability of psamosols for different crops and uses.

The soil profiles were deepened to approximately 150-200 cm. Each profile was characterized, establishing the following properties (Florea, 1987; 2003; Mihalache, 2006; 2009; Călina, 2023; Beek, 2000):

- the color of the horizons is determined using the Munsell Soil Color Charts, for wet and dry soil samples;
- the granulometric composition was determined by treating the soil with sodium pyrophosphate solution and separating the granulometric fractions by the wet sieving method (coarse sand), the siphoning method (fine sand) and the pipetting method, using the Kubiena mechanical pipette (dust and clay);
- the texture classes were established using the texture triangle;
- the density (D, g/cm^3), was determined by the pycnometer method, using the pump to remove air from the soil samples;
- the bulk density (BD, g/cm^3) was determined by the metal cylinder method, with a volume of approximately 100 cm^3 for soil samples collected in natural settings;
- total porosity (TP, %), was calculated with the relationship: TP, % = (1 – BD/D)/100;
- the maximum hygroscopicity coefficient (HC, %) was determined according to the Mitscherlich method, using a 12% sulfuric acid solution;
- the wilting coefficient (WC, %) was determined by calculation based on the maximum hygroscopicity coefficient, using the relationship: WC, % = HC x 1.5;
- the moisture equivalent (ME, %) was determined by centrifuging the wet soil sample at a centrifugal force of 1000 times the gravitational acceleration;
- the usable water capacity (UWC, %) was determined by calculation based on the moisture equivalent and the wilting coefficient, using the relationship: UWC, % = ME – WC;
- humus (%) was calculated by the Walkley-Black method, treating soil samples with concentrated sulfuric acid and potassium dichromate;
- total nitrogen (Nt, %) was determined using the Kyeldhal method;
- mobile phosphorus (P, mg/100 g soil) was determined by the Egner-Riehm-Domingo method, using a photocolorimeter;
- mobile potassium (K, mg/100 g soil) was determined by the Egner-Riehm-Domingo method, using a flame photocolorimeter;
- soil reaction (pH value) was determined by the potentiometric method, in 1/2.5 aqueous solution;
- calcium carbonate ($CaCO_3$, %) was determined using a Scheibler calcimeter, in percentages.

RESULTS AND DISCUSSIONS

1. Spread

The surface occupied by the sands and sandy soils on the left side of the Jiu has a triangular shape, with one side on the Jiu, another very sinuous one that starts from the place where the Amaradia flows into the Jiu, to the west of the Corabia locality and the third side runs parallel to the Danube in the direction of the localities of Sadova-Vădastra (Figure 1).

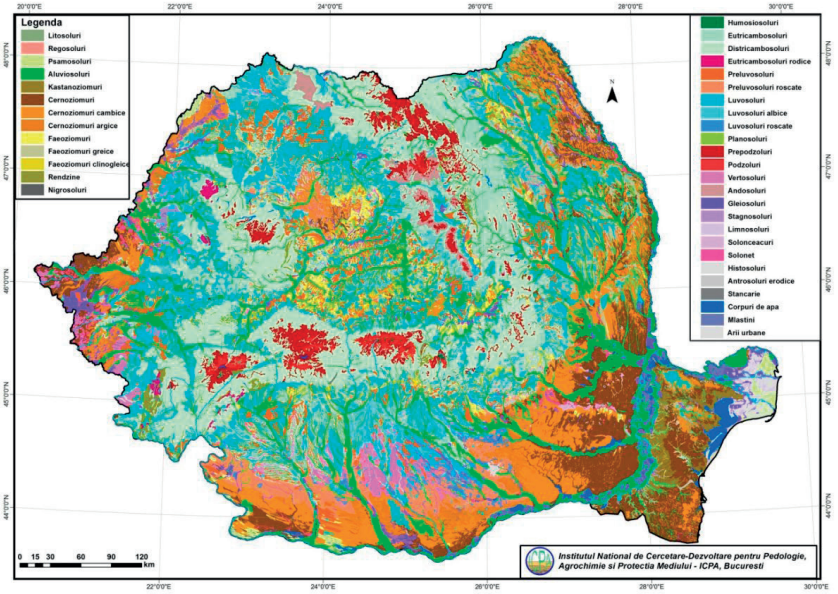


Figure 1. Soil map of Romania (according to ICPA Bucharest)

Of the total area of psamosols, approximately 80,000 ha are found between Jiu and Olt, being spread especially in the eastern plain of Oltenia, known as the "Romanați Plain" which is located south of the sinuous line Podari - Cârcea - Slatina (Canarache, 1990). The sands have a lacustrine origin and were Levantine deposits reworked by the Jiu River, which over time moved with its bed to the west. The sands were blown away by the dry winds from the southwest (Șorop, 1990).

The eastern plain of Oltenia which forms the southern part of the territory between Jiu and Olt constitutes, through its geological past, one of the important sectors of the Romanian Plain. It includes the central part "Leu - Rotunda Plain" as an extended tongue of the Getic plateau, surrounded by the terraces of Jiu to the west, of Olt to the east, and of the Danube to the south (Pop, 1971). All these terraces were formed in the first part of the Quaternary under the influence of the glacial epochs and interglacial phases that followed each other.

The terrace system on the left bank of the Jiu River is represented in the form of narrow strips arranged more or less regularly between the Jiu Plain and the Leu Field - Rotunda. The Jiu Terraces are connected to those of the Danube by a prolonged connection between the localities of Sadova and Listeava. From a lithological point of view, the terraces are made up of Quaternary deposits 10-20 meters thick, formed by loess or loessoid material deposited over layers of different thicknesses, of sand and gravel.

The sandy material on these terraces was probably brought by the waters flowing from the mountainous and hilly area, long before the Jiu River established its current bed. The action of scattering (wind shaping) of the sandy material with the formation of dunes and interdunes continues today more or less intensely on the entire sandy surface on the left side of the Jiu River. This section is favored by the relatively small and unevenly distributed precipitation during the year and by the high temperatures in

July and August, which sometimes reach over 60°C on the surface of the sands. The wind with a strong action of scattering the sands is the Austrul, which blows from the west. The Crivătul which blows from the north-east and east has a less scattering action.
The sand area between Jiu and Olt is presented as a set of longitudinal dunes, fixed or semi-mobile, oriented in the west-east direction, with heights of up to 8-12 m. The interdunes have very variable widths and can reach up to 500 - 800 meters wide.

2. Agropedological characterization of the mollic psamosol

Climatic conditions in the researched area

The climate with its component elements, precipitation, temperature, humidity, wind, is an active factor in soil formation, exerting both direct and indirect action. Direct action is manifested through the influence of the process of destruction of existing rocks, through physical degradation and chemical alteration, especially through climatic factors.
The southern area of Oltenia is characterized by a temperate-continental climate (C.f.a.x according to Koppen). The Mediterranean climate, which penetrates through the Danube gorge, has a strong influence on the southwestern part of the Romanian Plain, determining high average temperatures in the area, with milder winters and warmer summers. Thus, the multiannual average temperatures oscillate between 10.5-11.5°C, with higher values in the summer months (22-23°C) and lower values in the winter months, below 0°C (Table 1). It should be noted that in sandy areas, at the soil surface, the maximum temperatures increase significantly, reaching values of up to 50-60°C.
The physiological processes of plant organisms (respiration, photosynthesis, water and mineral circulation) are influenced at the same time by both air temperature and soil temperature. Temperature plays an overwhelming role on the development rate of different phases of plant vegetation. The multiannual average air temperature is 10.9°C in Craiova and 11.3°C in Bechet (Table 2).

Table 1. Multiannual and monthly average temperatures recorded at Craiova station

Period	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Average
1955-2023	-2.6	0.2	5.4	11.6	17.0	20.3	23.0	21.9	17.3	11.5	5.4	0.4	10.9

Table 2. Multiannual and monthly average temperatures recorded at Bechet station

Period	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Average
1955-2023	-2.9	0.6	5.6	12.2	17.8	21.0	23.1	22.3	17.8	11.6	6.3	0.7	11.3

Precipitation is a basic factor that ensures the accumulation of water reserves in the soil and a source of water supply for plants. The multiannual average is 545.4 mm at the Craiova meteorological station and 519.0 mm at the Bechet meteorological station. Calculating the moisture deficit and surplus for the localities of Craiova and Bechet, depending on the required precipitation, it resulted that in the months of July, August, September and October there is a moisture deficit ranging between 6.0 mm (July) and 20.1 mm (September), and the surplus is between 1.4 mm in April and 26.9 mm in December in Craiova.
The most frequent and strongest winds encountered in the Jiu meadow are those blowing from the west, the Austrul and from the east the Crivăţul. The Austrul is a dry wind that blows from the west and brings drought in summer and autumn. The Crivățul blows from the east and northeast, bringing snow and cold waves in winter, and rain in spring, sometimes dryness. Both winds, in spring when the land is not covered by vegetation, contribute to the scattering of sandy material from the dunes, forming real sandstorms.
Analyzing the climatic elements in the studied area as a whole, it can be seen that they fit into the general characteristics of the southern part of Oltenia, also preserving the microclimate aspect of the plain area. The regime of high temperatures and low precipitation combined with winds that blow frequently in this area throughout the year, especially in spring, summer and autumn, requires taking special

measures in order to ensure optimal conditions for plant development.

General data on the lithology of the sandy area

The surface lithology is represented by the aeolian reworked sandy and sandy-loamy deposits on the terraces (Anastasiu, 1998). Also on the terraces are areas whose lithology is represented by loess and loessoidal deposits with a clay-loamy-sandy texture. The Jiu terraces, lithologically, are made up of Quaternary deposits 10-20 m thick, formed by loess or loessoidal materials, deposited over layers of sand and gravel of different thicknesses.

The psamosols on the left side of the Jiu River were formed on sand and sandy materials brought by the Jiu River, deposited in the meadow and reworked by the winds. The quartz-sandy material is resistant to alteration processes, frequently occurring in the form of grains and shiny flakes. The intensity of alteration depends, in addition to the mineralogical composition, on the degree of humidity (Coteț, 1973; Dragoș, 1982; Florea, 2005). Thus, on dunes, the humidity being low, the alteration is barely noticeable, therefore the percentage of fine materials is low (below 5%). In interdunes, the humidity being higher, the alteration is more intense, and the percentage of fine material increases to 10-15%.

In general, the sandy parent material contains a percentage of 80-90% coarse material (sand) and a percentage of 10-20% fine material (dust and clay).

Hydrography

In the sandy area, the hydrographic network is extremely poor, and the groundwater is relatively deep. The sandy area on the left side of the Jiu River has practically no running water. The Jiu River, which receives a whole series of tributaries from the mountainous, sub-Carpathian and hilly areas, no longer has any tributary on the entire Craiova - Danube route.

The normal connection of the sands with the Jiu Meadow is interrupted by the sudden height of the terrace, which in the central part of the sands reaches over 50 meters above the Jiu River (Maxim, 1971). In the interval between Jiu and Olt, over 70 km long, the Danube does not receive any tributary from this sandy area. Within the mentioned area, however, there are some lakes formed from the water of coastal springs, such as the one from Bratovoiești - Prunet. A series of such springs were captured in the Gioroculuі Valley and constitute one of the sources of water supply for the city of Craiova.

The lack of a hydrographic network determines low values of relative air humidity in the dry season.

Hydrology

As for groundwater, its depth shows seasonal fluctuations depending on the rainfall, the terrain orography and the distance from the Danube. The groundwater level increases in winter and spring, due to higher rainfall and lower evapotranspiration, and decreases in summer and autumn, due to lower rainfall, intense evapotranspiration, high water consumption by plants, etc.

By monitoring the groundwater level at a number of 26 wells located between Georoc and Dăbuleni, it was concluded that the average water level varies between 5-13 meters, and at most of them (16 wells) the groundwater level is below 10 meters deep (Maxim, 1971). The groundwater level is different in the interdunes compared to the dunes.

So, in the Northern half of the researched area the groundwater depth is 5-10 m in the interdunes and 10-20 m on the dunes, and in the southern half the groundwater depth is approximately 2-3 m in the wide interdunes and 0.5-1.5 m in low areas on the terraces, and on the dunes we have 7-8 m.

Natural vegetation

It has a restricted area, due to the expansion of agricultural crops. It is also found in natural forest patches, not cleared, in the vegetation of ponds in this area, of natural meadows, in protective curtains and especially in the form of weeds in agricultural crops. Natural herbaceous vegetation is represented by various xerophytic species, characteristic of dry areas that have a weak development, especially on dune tops. As natural plants with a greater spread we mention the following: *Digitaria sanguinalis, Bromus tectorum, Cynodon dactylon, Trifolium arvense, Agropyrum repens, Vicia vilosa, Tragus racemosus, Trifolium campestre*.

Among the weeds found in agricultural crops with higher frequency are: *Delphinium consolida, Convolvulus arvensis, Cirsium arvensis, Portulaca oleracea, Aristolochia*

clematis, Setaria viridis, Sorghum halepense, Lepidium draba, Chenopodium album, Tribulus terrestris, Sambucus ebulus, Xanthium strumarium, Anthemis ruthenica, Euphorbia virgata, Ranunculus arvensis, Solanum nigrum.
Natural forests appear sporadically in this sandy area and are made up of acacia (*Robinia pseudacacia*) like those at Tâmbureşti, Mârşani, Malu Mare, Daneţi, or pedunculate oak (*Quarcus pedunculata*) and acacia like the forest at Ocolna. Among the shrubs, the following species appear: *Rosa canina* (rosehip), *Crataegus monogyna* (hawthorn), *Morus alba* (mulberry), *Pirus piraster* (forest pear), *Ligustrun vulgare* (dogwood).

Morphological description

Mollic psamosols are found in the interdunes and in the lower part of the dune slopes, where the humidity is higher. The more favorable humidity regime makes the vegetation develop better and leaves a greater amount of organic residues on the surface and inside the soil.
The presence of humidity over a longer period of time and in a greater amount determines more intense alteration and humification processes, therefore these soils are richer in humus and fine material (dust and clay). Mollic psamosols have a deeper profile, darker color, and the horizons are better differentiated from each other.
To characterize this soil, the morphological description and analytical data of a profile located in Dăbuleni are presented. The soil has an Am-AC-C type profile (Figure 2):
- Horizon Am: 0-55 cm thick, dark brown (10 YR 3/3) when wet and grayish brown (10 YR 5/3) when dry; sandy-loamy texture; glomerular structure, very friable; porous; loose; frequent filiform roots; gradual transition.
- Horizon AC: 55-87 cm thick; dark yellowish brown (10 YR 3.1/4) when wet, light yellowish brown (10 YR 4.5/4) when dry; sandy-loamy texture, unstructured, porous; medium compacted, rare filiform sprouts; gradual transition.
- Horizon C: under 87 cm thick: light yellowish brown color (10 YR 6/3) when wet and light yellow color (10 YR 7/3) when dry; sandy texture; unstructured; porous; medium compact; moist.



Figure 2. Soil profile

Physical-mechanical properties of soft psamosols

Interpreting the laboratory results (Table 3) it is found that the soil has a very high coarse sand content of 69.4% in the C horizon and varies along the profile, reaching 66.1% in the Am horizon. The fine sand content is lower and varies along the profile from 15.4% in the first horizon to 18.3% in the C horizon. The dust content decreases from 9.3% in the Am horizon to 5.8% in the C horizon. The clay content is also low, ranging between 9.2% in the Am horizon and 6.5% in the C horizon. Depending on the determined granulometric fractions, the soil texture is sandy-loamy throughout the soil profile.
The bulk density shows values that increase with depth, from 1.37 g/cm^3 in the first horizon reaching 1.50 g/cm^3 in the C horizon.
The density increases from 2.61 at the soil surface to 2.65 g/cm^3 in depth, this is due to the fact that at the soil surface the amount of organic material is higher. These values determine a lower porosity, varying on the profile from 48% at the soil surface to 44% in depth, which shows that the soil is strongly compacted.

Table 3. Physical-mechanical properties of mollic psamosols

Horizon	Depth (cm)	Granulometric composition, %				Textural class	BD	D	TP, %
		Coarse sand (2-0.2 mm)	Fine sand (0.2-0.02 mm)	Dust (0.02-0.002 mm)	Clay (<0.002 mm)		g/cm^3		
Am	0-55	66.1	15.4	9.3	9.2	NL	1.37	2.61	48
AC	55-87	67.2	16	8.1	8.7	NL	1.46	2.63	45
C	87+	69.4	18.3	5.8	6.5	NL	1.50	2.65	44

BD - Bulk density; D - Soil density

Hydrophysical properties of mollic psamosols

The hydrophysical indices (Table 4) show low values, which correlate very well with the humus and clay content. The hygroscopicity coefficient (HC) has high values in the first two horizons (1.8%) and very low values in the C horizon of only 0.7%.

Table 4. Hydrophysical properties of mollic psamosols

Horizon	Depth, cm	HC, %	WC, %	ME, %	UWC, %
Am	0-55	1.8	2.8	6.2	3.4
AC	55-87	1.7	2.6	5.4	2.8
C	87+	0.7	1.9	4.8	2.9

HC - Coefficient of hygroscopicity; WC - Wilting coefficient; ME - Moisture equivalent; UWC - useful water capacity

The wilting coefficient (WC) being calculated indirectly according to the hydroscopicity coefficient, presents the same variation on the profile from 2.8% in the Am horizon, up to 1.9% in the C horizon. The moisture equivalent (ME) determined in the laboratory by the configuration method, shows us that it decreases from 6.2% in the first horizon to only 4.8% in the last horizon.

The useful water capacity (UWC) has values ranging between 3.4% in the Am horizon and 2.9% in the C horizon.

Chemical properties of mollic psamosols

The data obtained from laboratory analyses (Table 5) indicate a much lower organic matter content in depth (0.34%) than at the surface (1.60%).

Table 5. Chemical properties of mollic psamosols

Horizon	Depth, cm	Humus, %	Nt, %	P_2O_5	K_2O	pH (H_2O)	HEC	SBs	CEC	BS, %
				me/100 g soil			me/100 g soil			
Am	0-55	1.60	0.070	7.1	11.1	6.2	1.0	8.3	9.3	89
AC	55-87	0.85	0.039	5.4	6.3	6.7	0.6	6.7	7.3	91
C	87+	0.34	0.028	3.2	4.5	6.9	0.5	5.2	5.7	91

HEC - hydrogen exchange capacity; SBs-Sum of bases; CEC-Cation exchange capacity; BS- Percentage of base saturation

The total nitrogen content (Nt) is low, oscillating along the profile from 0.070% in the first horizon to 0.028% in the C horizon. The mobile phosphorus content (P_2O_5) decreases from 7.1 in the Am horizon to 3.2 me/100 g soil. The potassium content is higher compared to phosphorus, namely in the first horizon, registering 11.1 me/100 g soil.

The pH value being 6.2 at the surface and 6.9 in depth shows that the soil reaction is weakly acidic. The sum of cations retained by the colloidal complex is low. The cation exchange capacity has values between 9.3 and 5.7 me/100 g soil. The percentage of base saturation (BS) is between 89 and 91%.

CONCLUSIONS

The sandy area on the left bank of the Jiu covers an area of approximately 80 thousand hectares and extends from Craiova to the Danube meadow. In the western part, the sandy area is delimited by the Jiu meadow and in the eastern part it is delimited by the Leu-Rotunda high field.

The sands on the left bank of the Jiu were formed by the erosion action of the waters of the Jiu River in the high area (mountainous and hilly) and the deposition of eroded materials in the meadow after which these deposits were rearranged by winds on the left bank.

The mollic psamosols are found in the interdunes where the humidity regime is better for plant cultivation and fruiting. They are better supplied with humus and nutrients and have a medium to low productive capacity. They have an Am-AC-C type profile, sandy-loamy texture and weakly acidic reaction.

Recommendations for agricultural practice:

- Psamosols are generally poor soils, so in order to be successfully cultivated, it is recommended to apply an agricultural technology specific to these soils.

- To complete the moisture deficit, it is recommended to apply irrigation. Since they retain little water, watering rates must be lower, applied at shorter intervals. In this way, water loss through infiltration is avoided and plants can benefit from a greater amount of moisture.
- Being poorly supplied with humus and nutrients, psamosols require permanent organic and mineral fertilization. Organic fertilizer is recommended to be buried at a depth of 20-40 cm to create a protective layer at this depth against the loss of water and nutrients in depth. It is also recommended to apply mineral fertilizers in more batches and at shorter intervals, to avoid their loss through leaching.

Sands and sandy soils, being non-cohesive soils, are permanently subject to the process of wind deflation. To reduce scattering, protective curtains, wetting irrigation, parasands, protective screens, etc. are used. Psamosols are soils with a coarse texture, do not retain water, are strongly aerated, poor in colloidal complex, therefore they require a specific cultivation technology for these soils.

In conclusion, mollic psamosol has somewhat better relations with water and air, is richer in colloidal complex, in humus and nutrients, for this reason it has somewhat higher fertility compared to other sandy soils. To improve mollic psamosols, a series of measures must be taken, including: deep loosening, enrichment in humus and clay, application of organic and chemical fertilizers, replenishment of moisture deficit through irrigation.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

BEHAVIOR OF WHEAT UNDER DIFFERENT TILLAGE SYSTEMS IN THE PEDOCLIMATIC CONDITIONS OF THE TRANSYLVANIAN PLATEAU

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Abstract

Modern agriculture's challenges are being addressed by implementing agricultural methods that improve the soil's condition and decrease CO_2 emissions. Through the research carried out at the Agricultural Research and Development Station Turda we aim to highlight, by comparison with the conventional system (CS- plough) the effectiveness of unconventional tillage systems (MTC- chisel, MTD- disk, NT- direct sowing) at winter wheat cultivation, in the soybean-wheat-maize rotation, from the perspective of technological implications. The experience is organized according to the subdivided plots method, with two replications. The biological material used in this experiment is the „Andrada" winter wheat variety. In the 11 vegetation periods (2013/2014……2023/2024), the average of winter wheat yield was highest in the MTC system (6,662 kg/ha), but with an increase in yield below 100 kg/ha compared to CS. Even though in the MTD and NT systems the yield is lower than in the CS, these systems, in addition to the benefits they have on the properties of the soil, also represent a way towards better economic efficiency. Sustainable development principles must be adhered to when adopting unconventional soil tillage systems, considering plant requirements and favorable climate and soil conditions.

Key words*: climate, fertilization, tillage system, wheat, yield.*

INTRODUCTION

The degradation of agricultural land, greatly intensified in recent years, represents a real problem worldwide. Anthropogenic activity contributes greatly to the worsening of soil properties, with negative effects on biodiversity, productivity, and food security (Lee, 2005; Drury et al., 2006; DeLong et al., 2015).

Numerous studies and research carried out, including Lehman et al. (2015), specify the need to implement immediate measures to contribute as much as possible to restoring soil health. Agriculture needs to adapt to new technological trends in the way the land is processed in order to set up crops (Karlen & Rice, 2015; Lal et al., 2020). Unconventional soil tillage systems are considered viable alternatives to the ploughing system (with furrow turning), protect the soil from erosion, improve the infiltration and conservation of rainfall water, effects mentioned by Cantero-Martínez et al. (2007), Moraru et al. (2010) and Cociu & Cizmaș (2015). Minimum tillage and no tillage technologies have expanded (Derpsh et al., 2010), and herbicides have taken a large part of the contribution of soil works in weed control, and here we refer to moldboard plowing, cultivators, disk harrow, rotating spading (Colbach & Cordeau, (2022). Importance of herbicides for no till agriculture in South America. Crop life. Retrieved from https://croplife.org/case-study/importance-of-herbicides-for-no-till-agriculture-in-south-america/).

These technologies can lead over time to obtaining productions close to those obtained in the conventional variant with plowing, but also to increasing the degree of flowering, with perennial species in particular (Berca, 2004; Chețan et al., 2022).

For the adoption of the unconventional soil work system, high-performance machines are needed, which have quite high costs (Chețan, 2019). Their use leads to significant fuel savings

(Kőller, 2003; Marin et al., 2015; Chețan & Chețan, 2020a), favorably influencing the cost of production (Tihanov & Ivanov, 2021).

Wheat belongs to the most cultivated cereals group in the world, according to Erenstein et al. (2022), the areas occupied by wheat being currently 217 million hectares. In wheat crop, one of the most important links in cultivation technology is weed control and is based primarily on knowledge of sources of soiling, biological characteristics of weeds, multiplication, spread, etc. (Tsyuk et al., 2021).

According to certain authors, in agricultural crops, dicotyledonous weeds predominate (Ionescu, 2011; Rusu et al., 2013; Stănilă et al., 2013; Steponavičienė et al., 2021; Manilov, 2022). In the wheat crop, the degree of enrichment is primarily due to the high reserve of seeds in the soil, but also to the fact that this plant has relatively low power to compete with weeds. A high degree of weeding can delay plant development and reduce the production quantitatively and qualitatively (Young et al., 1994; Harasim et al., 2014). There are various sources through which diseases and pests spread. For example: *Uromices pissi* thrives on *Euphorbia cyparissias*; and *Puccinia triticine* thrives on weeds from the *Gramineae* family (Pfunder et al., 2000; Bolton et al., 2008).

In recent years, climate conditions, characterized by high temperatures and low rainfall, have been less favorable to the successful establishment of autumn crops.

Due to unfavorable climatic factors, sowing was delayed in most areas of the country, resulting in an uneven growth of crops. This has led to a vulnerability to weed competition and sensitivity to pest attack. Among the autumn wheat varieties created in Turda is the 'Andrada' variety, characterized by an increased tolerance to environmental stressors, which, here we refer in particular to the temperature variations between night and day and the unevenness of precipitation (Moldovan et al., 2012).

The paper presents the results of the research carried out at the Agricultural Research and Development Station (A.R.D.S.) Turda, in the climatic conditions specific to the 11 agricultural years of experimentation (2013/2014…….2023/2024). The purpose of the study was to establish the influence of four types of soil tillage and two levels of fertilization on the winter wheat yield.

MATERIALS AND METHODS

The experimental field is located in the Western part of the Transylvanian Plateau, in the Southern part of Cluj County. The experiment was established on a Phaeozem soil (S.R.T.S, 2012), with clay-clay texture (M.E.S.P, 1987), pH slightly alkaline, medium supply level with total N and P, good supply with K, and moderate content in humus (Chețan & Chețan, 2020b). The crop rotation is three years, namely: maize-wheat-soybean. The biological material was the 'Andrada' variety.

The experimental factors were the following:

- A, soil tillage system with four graduations: conventional with plow (CS), minimum tillage with chisel (MTC), minimum tillage with disk (MTD), and no tillage with direct sowing (NT);
- B, fertilization level with two graduations: basic fertilization with $N_{48}P_{48}K_{48}$; basic fertilization with $N_{48}P_{48}K_{48}$ + in the spring when the wheat vegetation resumes with $N_{40.5}CaO_{10.5}MgO_{7.5}$;
- C, agricultural year with 11 gradations: 2013/2014…….2023/2024.

Wheat sowing in all tillage systems was done with the Directa-400 seeding machine; the sowing density was 550 germinating grains/$m^{2,}$ and the distance between the rows was 18 cm. The crop before winter wheat was soybeans, which were harvested relatively late; therefore, wheat sowing took place in the first decade of November of each experimental year. The particular importance of the technology of growing wheat, and not only, is the fight against weeds. In this experiment weed species were identified: annual dicotyledons (AD) like *Xanthium strumarium, Chenopodium album, Fallopia convolvulus, Polygonum arenastrum, Capsella rubella, Veronica persica, Sonchus oleraceus, Papaver dubium, Galeopsis bifida, Galium spurium, Consolida regalis, Myosotis arvensis, Stellaria media, Viola arvensis, Hibiscus trionum, Amaranthus retroflexus, Fumaria officinalis, Geranium dissectum, Galinsoga parviflora, Stachys annua, Anagallis arvensis*; perennial dicotyledons (PD) like *Convolvulus arvensis, Rubus caesius, Cirsium arvense, Taraxacum officinale, Rorippa*

armoracioides, Lathyrus tuberosus, Symphytum bohemicum, Achillea millefolium, Lepidium draba; annual monocotyledons (AM) like *Setaria helvola, Echinochloa hispidula, Avena fatua, Bromus tectorum* and perennial monocotyledons (PM) like *Elymus repens*.

Through the crop rotation practiced at A.R.D.S Turda, winter wheat follows the soybean crop (which is harvested quite late, in the first decade of November), thus, the number of weeds that appear in the newly established crop is quite small, and weed competition is reduced, herbicide application in autumn is not necessary. Account shall be taken of the observance of preventive measures, in the first place, crop rotation, the use of certified seeds, sowing data, sowing density, etc. These preventive measures are supplemented with chemical treatment at the end of the tillering phenophase of wheat, for controlling grasses and broad-leaf weeds. In this case, the treatment was carried out with 260 g/ha produced based on two active ingredients: florasulam (14.2 g/kg) + pyroxsulam (70.8 g/kg). To improve the effectiveness of the herbicide on weeds, 0.6 liters/ha of an adjuvant were applied.

The experimental field was harvested annually in the last decade of July, with the combine for harvesting experimental plots.

The yield was determined by weighing on the experimental parceling, after having eliminated the margins and transformed according to 14% STAS humidity (Cheţan et al., 2023).

The statistical analysis of the yield was performed using stability parameters based on the method proposed by Eberhart & Russell (1966) and also through cluster analysis using the Past4 Statistical Program.

Tables 1 and 2 present the climatic conditions recorded during the experimental period, in the Turda ARDS area measured by the Turda Meteorological Station (23'47'longitude; 46'35'latitude; 427 m altitude). The average value of multiannual temperatures recorded over a period of 67 years is 9.3°C, and that of precipitation is 532.4 mm. Regarding the thermal regime, it can be observed (Table 1) that in most agricultural years in which the study was conducted, with the exception of 2021, a warming trend in the average air temperature is observed; in some years, the 67-year multiannual average (9.9°C) was exceeded. In terms of the rainfall regime, higher amounts of precipitation, over 600 mm, were recorded in five years (2014, 2015, 2016, 2020, 2023); the excessively dry year was 2024 with only 389 mm (Table 2). Specific to the Turda area is the uneven distribution of precipitation. After long periods of drought, the rainfall that occur have a torrential character and are sometimes accompanied by strong winds and hail.

Table 1. Temperature in the agricultural years 2013/2014.....2023/2024 at the A.R.D.S. Turda

Month	Temperature (ºC) - agricultural year											
	2013/ 2014	**2014/ 2015**	**2015/ 2016**	**2016/ 2017**	**2017/ 2018**	**2018/ 2019**	**2019/ 2020**	**2020/ 2021**	**2021/ 2022**	**2022/ 2023**	**2023/ 2024**	**67 years**
October	11.2	10.8	9.7	8.3	11.6	12.7	13.5	12.0	9.7	12.4	14	**9.8**
November	7.1	5.7	6.1	2.9	4.9	6.0	8.9	3.2	4.4	5.4	5.2	**4.0**
December	-1.7	1.3	0.7	-2.7	1.0	-0.9	0.8	2.7	0.9	1.6	1.2	**-1.3**
January	0.5	-0.7	-2.8	-6.7	0.2	-2.2	-2.8	-0.6	-1	2.8	0.2	**-3.3**
February	3.8	0.6	4.6	1.5	-0.3	1.7	2.6	1.4	2.2	0.5	7.0	**-0.6**
March	8.8	5.5	5.9	8.4	3.3	7.3	6.1	3.3	3.6	6.3	8.8	**4.4**
April	11.4	9.6	12.4	9.9	15.3	11.3	10.3	7.8	8.8	8.8	13.3	**10.4**
May	15.1	15.8	14.3	15.7	18.7	13.6	13.7	14.1	16.3	15.4	15.8	**15.0**
June	18.5	19.4	19.8	20.7	19.4	21.8	19.1	19.8	21.1	19.0	21.7	**18.0**
July	20.4	22.2	20.5	20.3	20.4	20.4	20.2	22.7	23.1	21.8	24.0	**19.8**
Average	**9.51**	**9.02**	**9.12**	**7.83**	**9.45**	**9.17**	**9.24**	**8.64**	**8.91**	**9.4**	**11.12**	**9.3**

Table 2. The rainfall in the agricultural years 2013/2014.....2023/2024 at the A.R.D.S. Turda

Month	Precipitation (mm) - agricultural year											
	2013/ 2014	**2014/ 2015**	**2015/ 2016**	**2016/ 2017**	**2017/ 2018**	**2018/ 2019**	**2019/ 2020**	**2020/ 2021**	**2021/ 2022**	**2022/ 2023**	**2023/ 2024**	**67 years**
October	37.6	67.4	45.4	152.2	49.2	26.8	25.6	53.6	11.6	35.4	19.8	**35.4**

November	44.0	34.2	32	45.3	30.8	29.6	28.4	17.1	20.5	28.2	43.6	**28.2**
December	57.8	86.6	6.9	7.2	20.7	58.3	14.2	22.5	47.9	27.6	17.8	**27.6**
January	51.6	12.3	25	2.6	16.7	46	10.4	27	21.7	42.7	4.8	**21.7**
February	15.5	20.9	23.8	19.2	33.4	14.7	37.4	16.4	19.2	27.1	9.2	**19.2**
March	23.1	12.8	47	46.1	40.9	12.3	34	27.3	24.2	10.8	37.7	**24.2**
April	72.0	32.2	62.2	65.2	26.2	62.6	17.8	38.4	45.6	30.5	38.8	**45.6**
May	66.2	66	90.4	65.4	56.8	152.4	44.4	80.8	69.4	33.2	60.7	**69.4**
June	48.4	115.7	123.2	30.6	98.3	68.8	166.6	45.0	84.6	144.5	36.2	**84.6**
July	144.4	52.2	124.9	110.2	85.7	35	86.8	123.1	78	85.8	49.3	**78.0**
Sum	**560.6**	**500.3**	**580.8**	**544**	**458.7**	**506.5**	**465.6**	**451.2**	**422.7**	**465.8**	**317.9**	**532.4**

RESULTS AND DISCUSSIONS

The yield capacity of the winter wheat, „Andrada" variety, demonstrated considerable variation, being influenced by the two technological factors studied: the soil tillage system and the level of fertilization. As can be seen from Figure 1, throughout the 11 years of experimentation, average yields ranged from 6,156 kg/ha, achieved with the no-tillage (NT) system and basic fertilization, to 6,666 kg/ha, obtained under the conventional plowing system with additional fertilization.

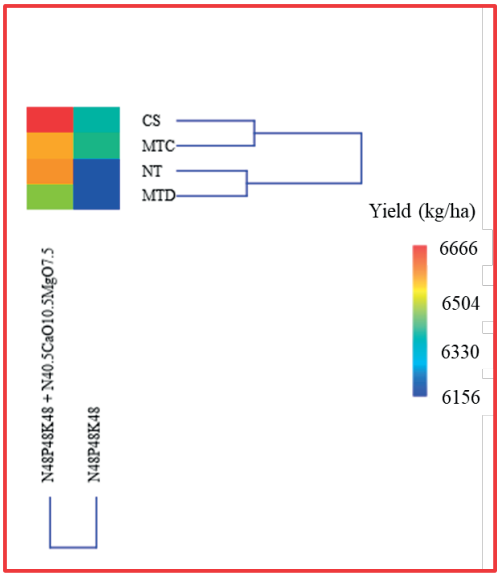


Figure 1. The yield potential of the winter wheat, based on the soil tillage system and fertilization levels

The result clearly indicates the significant impact of fertilization on wheat yield, with higher yield observed in treatments where additional fertilization was applied, regardless of the soil tillage method. Regarding the tillage system, the conventional system with plow (CS) and minimum tillage with chisel (MTC) yielded higher results compared to the other two tillage variants (MTD, NT). Similar results were reported by Chiriță et al. (2023), who pointed out that the higher yield was obtained in CS (7,148 kg/ha) and in MTC (6,662 kg/ha). It also mentions that in MTD and NT systems, the yield was 533 kg/ha, respectively 633 kg/ha lower compared to the CS variant.

The results obtained by Gandía et al. (2021), in an experiment with three tillage systems (conventional, minimum, no-tillage) in the 2014-2017 period, highlight the fact that the soil cultivation system did not significantly influence the wheat yield, the greatest influence being attributed to the precipitation regime.

When analyzing the influence of the year and the soil tillage system on the wheat yield obtained (Figure 2), it is evident that climatic conditions played a significant role in achieving high yields, regardless of the tillage system used.

The years 2014, 2015, and 2016 allowed the studied genotype to reach the highest yield in the experiment, exceeding 7,200 kg/ha obtained in both the CS and the MTC. Conversely, the year 2022, characterized by adverse thermal and hydric conditions, resulted in a decline in yield, with a reduction to approximately 3,500 kg/ha (MTD and NT).

Yields above 6,000 kg/ha were recorded in the conventional plowing system during the 2017, 2019, and 2024 growing seasons. The heat map analysis further illustrates that the year had a more substantial effect on yield variation than the technological factor of the soil tillage system.

The analysis of the interaction between fertilization and the crop year on grain yield for the winter wheat emphasizes the critical role of climatic conditions in achieving substantial yield increases, alongside the influence of fertilization practices.

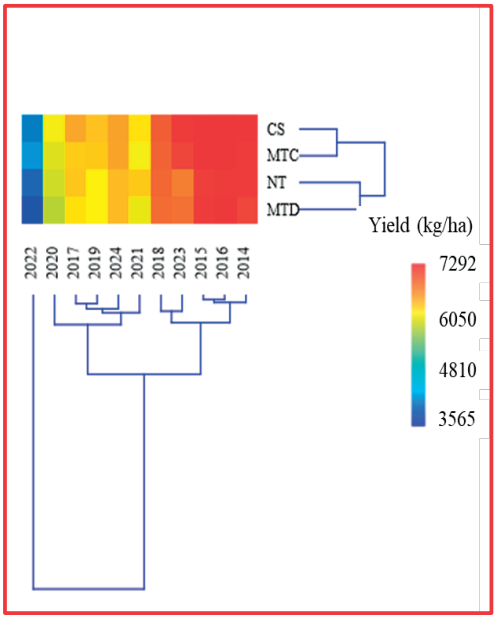


Figure 2. The yield potential of the winter wheat, based on the soil tillage system, in 11 different agricultural years

The additional fertilization resulted in higher yield across all experimental years.

Notably, the lowest yield (4,022 kg/ha) was observed in 2022, under treatment with only base fertilization. The highest yield, 7,402 kg/ha, was recorded in 2015, when supplemental fertilization was applied, as can be seen from Figure 3.

Based on the analysis of the production stability of the winter wheat, variety 'Andrada', over an 11-year period, considering different soil tillage systems and fertilization levels, it is evident that the conventional plowing system (CS) demonstrates superior performance under favorable environmental conditions, while the minimum tillage system with chiseling (MTC) shows higher values than plowing under unfavorable environmental conditions. Although yields from minimum tillage (MTD) and no-tillage systems (NT) are slightly lower, they remain acceptable, exceeding 6,400 kg/ha in favorable environmental conditions. anticipated, higher nutrient input results in greater yields compared to baseline fertilization (Figure 4).

From the study conducted by Aula et al. (2023) in the long-term experiment (initiated in 1970) regarding the grain yield under different tillage systems (no tillage, stubble mulch, moldboard plow), results show a more stability yield in the stubble mulch tillage system compared with no tillage and moldboard

plow. Contrary, Seddaiu et al. (2016) and Xu et al. (2019) showed that no tillage may lead to a more stable grain yield than the conventional tillage system.

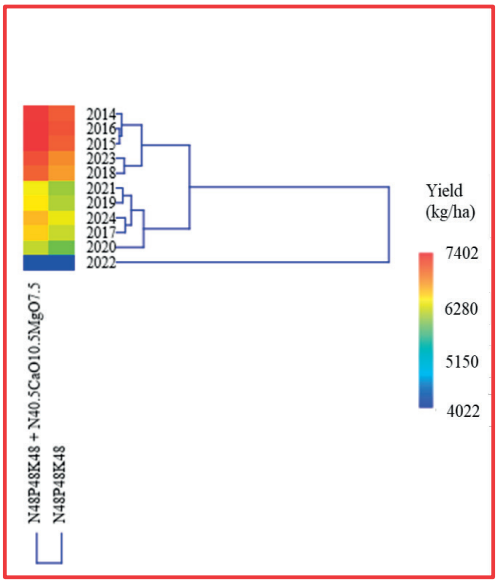


Figure 3. The yield potential of the winter wheat, based on fertilization levels, in 11 different agricultural years

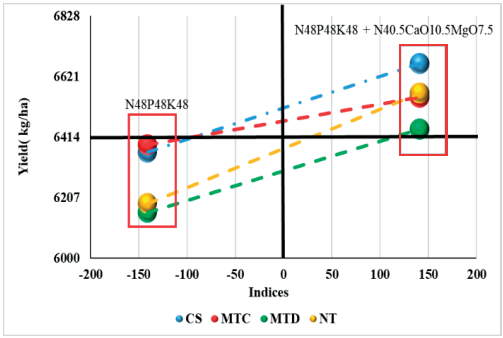


Figure 4. The yield stability of winter wheat, based on fertilization levels and tillage system, in 11 different agricultural years

CONCLUSIONS

Specific to the 11 years of experimentation was the uneven distribution of precipitation and high temperatures; in the specific area of the Transylvanian Plateau, production is closely

linked to the climatic conditions of the vegetation period.

Tillage has a lower impact on wheat production, with production differences of almost 200 kg/ha, indicating that unconventional systems can be an alternative to the conventional tillage system. Additional fertilization, against the backdrop of meteorological changes in most experimental years, did not lead to substantial increases in production; the results obtained can contribute to the existing data in the specialized literature on winter wheat cultivation technologies in the Transylvanian Plateau.

The conventional tillage system (CS) remains a viable option in terms of soil cultivation, achieving higher yields under favorable environmental conditions, while the minimum tillage system with chisel plow (MTC) records higher production values under unfavorable environmental conditions.

Although yields from the minimum tillage (MTD) and direct seeding (NT) systems are slightly lower, they remain acceptable, exceeding 6,400 kg/ha under favorable environmental conditions.

ACKNOWLEDGEMENTS

This work was supported by the Ministry of Agriculture and Rural Development, ADER project financing contract no. 123/17.07.2023 "Conservation of soil resources by using the technological components of regenerative agriculture in order to obtain economic and sustainable harvests of grassy cereals in the Transylvanian Plateau".

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

ORGANIC AGRICULTURE HAS DEFECTS IN THE SCOPE OF MINERAL FERTILIZATION

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Abstract

The organic agriculture system refuses mineral fertilizers in a wide range; however, plants utilize essential plant nutrients in mineral forms. This is because the elements bonded to the structure of organic matter are not readily available for plant uptake. Thanks to the mineralization process that leads to mineral nutrients released from organic matter, the decomposition rate depends on several environmental factors. Thus, either lower or higher nutrient amounts may appear in the soil which would be far from meeting plant nutrient requirements or ecological concerns may arise. Nitrogen has a priority in the scope of environmental issues. The results gathered by the working group revealed that nitrate concentration may be even higher in organic farming systems on average. Some researchers draw attention to mineral fertilizer production's economic and environmental costs. Yet, ammonia gas sources in the livestock industry threaten the atmosphere and may be sources of organic agriculture-friendly nitrogenous fertilizer. This article was prepared to highlight that using mineral fertilizers from certain sources should be allowed in organic agriculture within certain limits and based on soil analysis.

Key words*: organic agriculture, global warming, ammonia emission, food security.*

INTRODUCTION

The organic farming system, which appears as a new concept, refers to the agricultural system before the discovery of pesticides and fertilizers. In other words, organic farming is not an innovative system but a return to pre-discovery of agro-biocides and mineral fertilizers.

The priority in the organic farming system is to establish a healthy soil-plant-environment system without harming the environment and preventing the misuse of inputs (Bello, 2008). But the yield obtained from areas where organic farming techniques are applied is generally lower than in areas where conventional farming is done (Reddy, 2010; Röös et al., 2018). Therefore, revolutionary developments are required in today's organic farming system to improve yield. Yet, in today's organic farming concept, there are strict prohibitions on mineral fertilizers containing macro elements, especially nitrogenous fertilizers. However, renewable nitrogen sources should be developed and included in the system (Röös et al., 2018). While many producers want to shift to organic farming for economic reasons, research has shown that in some cases, inputs may be even higher than in conventional production (Cacek & Langner, 1986). Although the positive effects of organic farming are often mentioned, organic farming methods also have some negative environmental effects.

These effects include reduced soil fertility due to the decrease in the soil's nutrient pool, possible increases in soil acidity (Conacher & Conacher, 1998). There is also evidence that organic farming areas are not very safe in terms of nitrate pollution, which is the biggest concern in the conventional farming system (Erol et al., 2010). Similar concerns also arise in the livestock sector.

The effects of organic farming on livestock are also unclear, and it is difficult to find data that would allow a definitive judgment on this issue (Cabaret, 2003).

Veisi et al. (2017) reported that the motivation for organic farming is environmental protection, increasing farmers' income, and reducing input costs, while social ethical rules are less important. Therefore, additional measures to alleviate economic concerns may encourage producers planning to switch to organic farming. The measures taken could also alleviate concerns that organic farming will not meet the

requirement of the rapidly growing population (Jouzi et al., 2017) worldwide.

There are basically two reasons for the prohibition of the use of nitrogenous mineral fertilizers in organic farming: (1) to prevent the presence of high amount of mineral nitrogen in the soil instantly and the environmental concerns that excess mineral nitrogen will cause, and (2) the high energy input used during the production of mineral fertilizers and the environmental pollution that occurs during production. This study includes explanations that will eliminate both concerns. In summary, this study aimed to compile some of the studies existing in the literature and to reveal that allowing the controlled use of nitrogenous mineral fertilizer such as nitrate and ammonium in the organic farming system can provide both economic and environmental benefits.

Obtaining environmentally friendly nitrogenous fertilizer

It is thought that an environmentally friendly nitrogenous fertilizer production is possible based on the patented ammonia trap design (Coşkan & Atılgan, 2018). In other words, nitrogen fertilizers obtained from the ammonia trap system do not conflict with the philosophy of organic agriculture. In the ammonia trap system (Figure 1) polyethylene balls are placed in a closed barrel to obtain a high surface area. Then, 6N phosphoric acid solution is added to the barrel and the balls are continuously wetted with the acid solution under the system via a small magnetic pump. At the same time, the barn atmosphere is passed over the balls with the help of a small-scale kitchen fan. As a result, the ammonia gas, which is a base, reacts with the acid and allows the ammonium phosphate fertilizer to be obtained (Coskan & Atilgan, 2018). The appearance of the system developed by Atilgan et al. (2018) is shown in Figure 1. The protection of the utility model with the patent number TR201213292 (Anonymous, 2026) has already been expired.

Mineral fertilizers are commonly produced by the Haber-Bosch method, in which ammonia is obtained from nitrogen and hydrogen gas under high temperature and pressure. This production is responsible for 1-2% of the global greenhouse gas emissions (Lin et al., 2023). However, it is possible to obtain nitrogenous mineral fertilizers from rather less available natural sources in the environment, in limited quantities, without causing environmental pollution. One of these sources is ammonia gas released into the atmosphere in broiler barns (Coskan & Atilgan, 2012). It is possible to convert this gas into nitrogenous mineral fertilizer by capturing it with an acid that is allowed for use in organic farming, such as phosphoric acid. In this way, far from polluting the environment, the health of the personnel working in the poultry house can be protected while reducing atmospheric pollution according to the equation below (Coskan & Atilgan, 2018):

$$NH_4OH + H_3PO_4 \rightarrow NH_4H_2PO_4 + H_2O$$

The bound $NH_4H_2PO_4$ is an ammonium phosphate, which is frequently used in agricultural production as a mineral fertilizer. By obtaining this fertilizer via this process, both the contribution to global warming can be reduced and the quality of the air inside the barns can be increased.



Figure 1. Patented ammonia trap system (Atilgan et al., 2018)

The changes in ammonia concentration inside the barn after activation of the ammonia trap system are presented in Figure 2. As seen from Figure 2, the overall ammonia level in the broiler barn has reduced significantly over time.

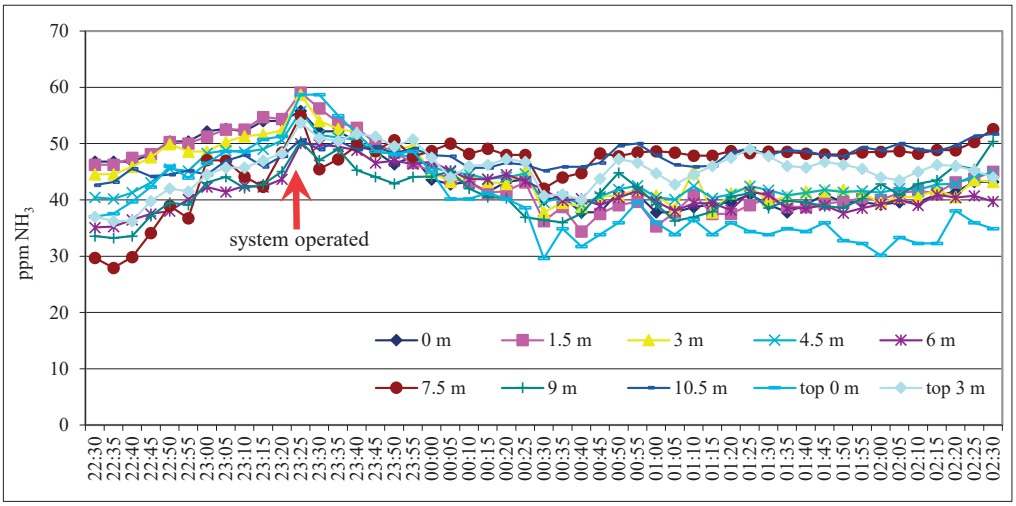


Figure 2. Ammonia reduction by ammonia trap (Atilgan et al., 2018)

Thus, obtained fertilizer can be compatible with the philosophy of organic agriculture since it uses very little energy compared to the Haber Bosch method and reduces environmental pollution. It is obvious that if this fertilizer is allowed to be used in organic agriculture within certain limits, it can be produced in animal breeding farms, and thus the profitability of these enterprises can be increased.

Although it is obtained while protecting the environment and provides multiple benefits, this fertilizer is not allowed to be used in organic agriculture. In conventional agriculture, it would not be wrong to say that mineral fertilization, which is carried out based on soil and plant analyses and is aware of all reactions that the given fertilizer will be involved in, will have a minimal negative impact on the environment and human health. Moreover, even under strict organic farming rules, the excess amount of mineral nitrogen may be abundant in the soil. Agricultural pesticides and synthetic plant growth regulators are excluded from this assessment. However, organic fertilization should look favourably on such new approaches in terms of mineral fertilizers.

Soil mineral nitrogen content in organic and conventional farming systems

The philosophy of organic farming emphasizes that the use of nitrogenous mineral fertilizers can cause an increase in mineral nitrogen forms in the soil. In order to test this view, soil samples were collected from 7 conventionally and 12 organically farmed areas. Nitrate, nitrite, and ammonium contents of these samples were determined. In addition, biological activity parameters and organic matter, and microbial biomass carbon analyses were also performed on the samples. However, only mineral nitrogen amounts were used in this study (Erol et al., 2010).

The soil mineral nitrogen contents in conventional and organic farming systems are presented in Figure 3 as mean values.

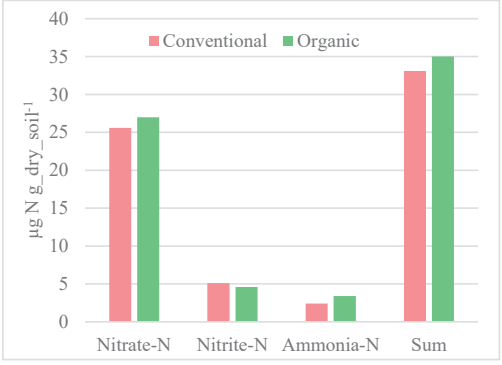


Figure 3. Nitrogen contents of conventional and organic farms (Erol et al., 2010)

As seen in Figure 3, while nitrite content was found to be slightly lower in conventional agriculture, areas where organic farming was performed had higher levels in other forms and total mineral nitrogen. This result is not

consistent with studies such as Bello (2008), Reddy (2010), Röös (2018), and others not cited here, which report that organic farming is less risky for the environment. This situation is most likely related to the inability to control at what speed and over what time the organic fertilizers used will mineralize. It is possible that organic matter that remained from previous years and could not be mineralized due to unsuitable conditions can also be mineralized rapidly under suitable conditions. The fact that more organic matter was determined in areas where organic farming is carried out (Erol et al., 2010) supports this idea. Therefore, it is difficult to manage nitrogen that may come from organic matter, and this has been demonstrated by this study. Based on the results of this study, it can be said that by slightly reducing the dose of organic fertilizer, the use of slow-release nitrogenous fertilizers produced with environmentally friendly methods, in case of a possible deficiency, can prevent instant high nitrate formation in the soil, and further research on this subject is required.

Plants-available N forms and plant nitrogen uptake

While plants can take up nitrogen as low molecular weight compounds such as nitrate, ammonium, and amino acids, complex organic compounds are not directly available to the plants (Paungfoo-Lonhienne et al., 2008). Kacar (2012) reported that plants receive 95% of the nitrogen they need in the form of nitrate and/or ammonium, while the remaining 5% can be obtained in the form of various organic compounds, including urea. In general, it is seen from these studies that it is not possible to provide adequate plant nutrition with organic fertilizers alone without the mineralization process. When viewed from this perspective, the fate of nitrogen provided by organic fertilization depends on the mineralization process, which is hard to predict its rate. Therefore, it is quite difficult to control the amount of mineral nitrogen originating from organic matter in the soil. In conclusion, if plants receive a significant portion of the nitrogen they need in mineral form and if it is difficult to control mineral nitrogen in the soil in organic farming (Erol et al., 2010), the controlled use of mineral nitrogen should be discussed before it is rejected outright.

Mineralization rates of organic fertilizers

The rate of mineralization of organic matter depends on many factors, and organic matter from different sources mineralizes at different timescales (Geisseler et al., 2021). The behaviour of some organic matter sources in the soil may be more attractive than others. For instance, if chicken manure is applied together with other fertilizers, an increase in the rate of mineralization may occur (Ribeiro et al., 2010). Cassity-Duffey et al. (2020) reported that nitrogen mineralization occurs over a wide range depending on the type of organic matter used. The researchers added new information to the literature by showing that commercial organic fertilizers are rapidly mineralized immediately after application and that organic fertilizers, which have been considered slow release until now, may not be the case. More interestingly, repeated long-term organic matter applications further increase the mineralization rate. Going one step further, it has been shown that repeated organic matter applications increase the mineralization rate even more (Zhang et al., 2012).

From the information in the literature, it has been observed that the mineralization rate and, therefore, the amount of mineral nitrogen in the soil are affected by many factors. Temperature and humidity have been shown to have strong effects on the release of nitrate and ammonium from organic matter (Cannavo et al., 2022). Therefore, generalizing on mineral nutrient release will not always yield precise results. It has been observed that giving nitrogen in organic form does not instantly reduce the presence of mineral nitrogen in the soil and may even increase it. Considering this information, it is open to debate whether organic nitrogen should be given instead of mineral nitrogen to protect the environment.

CONCLUSIONS

The reaction against mineral fertilization is due to a simple illusion. Because plants use nutrients in mineral form. The elements in the organic structure of organic fertilizers incorporated into the soil are not usable by plants in a wide range. When farm manure, harvest residues or green manure are applied, the plant nutrients they contain are converted into forms that can be used

by plants through a process called mineralization (Gök et al., 1998, 1999). The rate of mineralization depends on many environmental factors and can cause the presence of elements in the soil that are sometimes insufficient that threating plant nutrition and sometimes excessive threating the environment. This situation is an indication that the amount of mineral nitrogen in the soil cannot be controlled by organic fertilization. On the other hand, the rule that the elements needed by plants should be present in the soil at the time they are needed can be provided to a limited extent by organic fertilizer applications. Another important issue is that organic matter should be mixed into the soil, therefore, it shows that dressing fertilization can be done to a limited extent, especially in plant cultivation where the soil surface is completely covered. In other words, it can be said that the entire organic matter application should be made before planting the seeds. Organic farming advocates claim that the organic matter given at the beginning gradually provides the nutrient elements in parallel with the plant's needs. However, it is also thought that this may not be exactly the case (Cassity-Duffey et al., 2020). Zhang et al. (2012) reported that repeated organic matter applications further increased mineralization. This may be the factor responsible for the high nitrate abundance of organic farming sites' soils, as Erol et al. (2010) reported. It should be noted that long-term mineral N applications accelerate the mineralization of the soil's permanent humus and may threaten soil health in this respect. General information has revealed the negative effects of mineral nitrogen fertilizers on the environment. However, this situation is not related to whether mineral fertilizers are used, but to the dosage. Therefore, it is thought that the negative effects of well-adjusted mineral fertilizers on the environment may be the same or even less than organic fertilizers. In addition, organic fertilization accelerates the formation of nitrate in the soil via the nitrification process (Zhang et al., 2012).

Organic farmers also face challenges such as pest and weed control when farming in areas previously farmed using conventional methods (Conacher and Conacher, 1998). In this study, only the situation in terms of mineral substances was evaluated. If reliable, environmentally friendly chemical solution proposals for pest and weed control emerge, it should be evaluated whether allowing the use of these substrates can be a tool to meet the needs of the increasing population.

As a result, (1) plants can mainly uptake nutrients in mineral forms, (2) more nitrate may accumulate in organic farming areas and this can threaten the environment, (3) mineral nitrogen fertilizer can be obtained with environmentally friendly methods, (4) allowing the use of mineral nitrogen in organic farming, even if limited, can eliminate concerns about yield loss. The main idea that is intended to be conveyed in this study is that if the controlled use of mineral fertilizers is allowed in organic agriculture, the decrease in yield experienced in organic agriculture can be prevented while protecting the environment.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

A COMPARATIVE STUDY OF THE BENTONITES AND NATURAL ZEOLITES CAPACITIES TO REDUCE THE BIOAVAILABILITY OF HEAVY METALS IN A CONTAMINATED SOIL

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Abstract

Soil contamination with heavy metals is a major environmental challenge, particularly in industrial regions such as Copșa Mică, Romania. Past industrial emissions have resulted in substantial soil pollution, with levels of lead (Pb), cadmium (Cd), and zinc (Zn) exceeding permissible limits. This study investigates the effectiveness of various bentonites and natural zeolites in reducing heavy metal mobility and bioavailability in contaminated soil. Laboratory incubation tests were conducted using six inorganic additives sourced from Romania. The additives were applied at a 3% (w/w) dosage, and their effectiveness was evaluated using single metal extraction methods with DTPA + $CaCl_2$ + TEA (pH 7.3) and 1M NH_4NO_3. The results demonstrate that all amendments significantly reduced heavy metal mobility, with variations depending on the specific metal. Activated bentonite exhibited the highest efficiency in immobilizing Pb and Zn, while zeolite proved most effective for Cd stabilization. This study highlights the potential of natural zeolites and bentonites as cost-effective and environmentally friendly solutions for soil remediation in heavily polluted areas.

Key words*: additives, contamination, immobilization, metals, soil.*

INTRODUCTION

Soil pollution with heavy metals is a global environmental issue, directly affecting ecosystems, agriculture, and human health. Heavy metals such as lead (Pb), cadmium (Cd), and zinc (Zn) are highly toxic and persistent in the environment, tending to accumulate in soils, where they reduce fertility and can be absorbed by plants, entering the food chain (Bolan et al., 2014; Buzatu & Dodocioiu, 2023). In Central and Eastern Europe, industrial regions are particularly affected by this issue, especially those with a long history of intensive industrial activities and insufficient environmental regulations in the past.

In Romania, Copșa Mică stands as a stark example, with decades of industrial emissions from SOMETRA and CARBOSIN leading to widespread heavy metal pollution. According to Vrînceanu et al. (2010), the area impacted by this pollution reached approximately 180,000 hectares by the 1990s. This contamination, exacerbated by the region's acidic soils, necessitates immediate remediation to mitigate risks to human and environmental health.

This area requires immediate interventions to reduce the mobility and bioavailability of metals. Soil in this region, which are generally acidic, favor increased mobility of heavy metals, amplifying the risks associated with their transfer into the food chain. Increasing the soil pH through inorganic amendments such as zeolites and bentonite can significantly reduce the mobility of metals, contributing to the protection of the environment and human health (Ören & Kaya, 2006).

Heavy metal contamination is increasingly becoming a significant threat to soil quality worldwide (Houben et al., 2013; Cui et al., 2016, cited by Vrînceanu et al., 2019). Furthermore, their absorption by plants represents a source of food contamination with severe effects on human health, including neurological, renal, and cardiovascular problems. Moreover, soil contamination with heavy metals is also a major environmental problem, having a profound impact on human

health, groundwater quality, and agricultural productivity. The main sources of these contaminants include industrial activities, uncontrolled waste discharges, and excessive use of chemical fertilizers. Heavy metals such as lead (Pb), copper (Cu), cadmium (Cd), and zinc (Zn) are particularly dangerous due to their persistent toxicity and ability to bioaccumulate in the food chain (Alloway, 2013; Liu et al., 2022).

Remediation of soils polluted with heavy metals is a priority in the context of environmental protection and public health. Due to their persistence in the environment and toxic effects, managing these pollutants requires efficient and accessible technologies. Therefore, developing effective, ecological, and economically accessible remediation methods is essential. Conventional solutions, such as excavating and storing contaminated soils, are expensive and have a negative environmental impact.

Numerous specialized studies have highlighted the effectiveness of using inorganic additives in immobilizing heavy metals. In fact, two key properties of these materials are exploited: the alkaline reaction and/or their high cation exchange capacity.

The effects of incorporating materials with a high metal retention capacity into the soil are amplified under alkaline conditions. This is due to the increase in the number of metal retention centers, intensifying retention processes and reducing the number of competing protons for the sorption sites in the soil's adsorptive complex (Vrînceanu et al., 2010).

Heavy metal immobilization is a remediation method aimed at reducing the mobility and bioavailability of these metals, without completely removing them from the contaminated environment. This method involves using additives that stabilize heavy metals through physicochemical mechanisms such as adsorption, ion exchange, or precipitation. Among the most promising additives are bentonite, a clay with remarkable colloidal properties, and natural zeolite, an aluminosilicate material with a porous structure. The specialized literature (De Compos et al., 2010) has identified 40 natural zeolites and over 150 synthesized zeolites, according to Pant et al. (2020). The main objectives of these amendments are to sequester heavy metals through chemical, physical, and biological processes, reducing their mobility and bioavailability. A secondary objective of the amendments is to stabilize pollutants when the amendments can form stable compounds with pollutants, preventing them from spreading into the environment. A third goal would be to reduce the mobilization capacity by modifying the pH, soil structure, or organic matter content. Some researchers argue that bentonite is known for its ability to adsorb heavy metals through stable chemical bonds, while zeolites offer increased ion exchange potential due to their large surface area and high porosity.

This research aims to evaluate the capacity of natural zeolites and bentonites to immobilize heavy metals in a contaminated soil sample from the Copșa Mică area, providing a scientific basis for implementing effective remediation measures. Through a laboratory experiment, the efficiency of these materials in reducing the mobility of heavy metals in contaminated soils will be analyzed, offering sustainable solutions for their remediation.

This research contributes to understanding the mechanisms involved and the development of ecological technologies applicable in areas affected by heavy metal pollution. The need to restore the initial properties of the soil has driven us to search for new and reliable alternative soil cleaning methods.

MATERIALS AND METHODS

Study area and soil sampling

The study was conducted in the Copșa Mică area, one of the most polluted regions in Romania, located in the Transylvanian Depression. Soil pollution in this area is characterized by high concentrations of heavy metals, such as lead, cadmium, and zinc, which exceed the permissible limits for agricultural or residential uses.

For this study, the soil material used was collected from the vicinity of the former SOMETRA industrial platform, approximately 1,775 meters from the factory's exhaust stack, at a depth of 0-20 cm, corresponding to the arable layer that was affected. The collected material was dried, crushed, homogenized, and

passed through a 2 mm sieve to remove roots, stones, and other impurities.

Soil characterization

The physicochemical properties of the soil were analyzed at the National Institute for Research and Development in Soil Science, Agrochemistry, and Environmental Protection (ICPA Bucharest). The results, presented in Table 1, revealed heavy metal concentrations (Cd, Cu, Pb, and Zn) exceeding the intervention thresholds for sensitive land uses as per Order 756/1997. The soil's acidic pH (4.59) and medium texture further contribute to the high mobility of these pollutants.

Table 1. Average values of the main chemical and physical properties of the soil material

Characteristic	Unit of measurement	Average value (n=3)
pH		4.59
Kjeldahl Nitrogen content	%	1.119
Organic Carbon content	%	1.06
Available Phosphorus Content (PAL)	mg/kg	16.7
Available Potassium content (KAL)	mg/kg	119
Cadmium content (Aqua Regia)	mg/kg	35.1
Copper content (Aqua Regia)	mg/kg	206
Lead content (Aqua Regia)	mg/kg	2,815
Zinc content (Aqua Regia)	mg/kg	2,630
Cadmium content (NH_4NO_3)	mg/kg	27.0
Copper content (NH_4NO_3)	mg/kg	11.88
Lead content (NH_4NO_3)	mg/kg	616
Zinc content (NH_4NO_3)	mg/kg	1,257
Textural Class	SM - Medium Texture – Medium Sandy Clay	

Inorganic additives

Comparative studies of various immobilization materials often employ a methodology that includes percolation experiments, bioaccessibility testing of metals, and examination of factors such as temperature, contact time, type of metal, and environmental pH to evaluate immobilization efficiency (Vrînceanu et al., 2010).

This study investigates the capacity of two inorganic additives, bentonite and zeolite, to immobilize heavy metals in soil. These additives have garnered significant interest in the scientific community for their potential in environmental protection. A brief characterization of these inorganic additives used in the case study, according to the technical sheet provided by the manufacturer, is presented in Figure 1.

Incubation experiment

To evaluate the effects of various additives on heavy metal immobilization, incubation tests were conducted in the Physico-Chemical Analysis Laboratory of the National Institute for Research and Development in Soil Science, Agrochemistry, and Environmental Protection (ICPA) in Bucharest. The experiment focused on a single factor: the type of additive used.

Six different treatments were evaluated in this study, each using a different additive to immobilize heavy metals:

- *Bentonite-based additives*

a1 – B_{ha}GS - bentonite for animal feed (Gurasada, Hunedoara County)

a2 – B_{nac}GS - non-activated ground bentonite (Gurasada, Hunedoara County)

a3 – B_{acff}GS - activated bentonite ground with drilling fluids (Gurasada, Hunedoara County)

- *Zeolite-based additives*

a4 – $Zn_{(0\text{-}0.4)}$ZL – natural zeolite (0-0.4 mm) (Zalău, Sălaj County)

a5 – $Zn_{(1\text{-}3)}$ZL - natural zeolite (1-3 mm) (Zalău, Sălaj County)

a6 – ZnRP - natural zeolite from Rupea (Rupea, Brașov County)

Each additive was applied to the soil at a concentration of 3% of the dry soil mass. A control group with no additive was also included in the experiment.

For the incubation experiment, approximately 1 kg of dry soil material was weighed into each of the 21 boxes. After incorporating the equivalent dose of 3% of each additive into the contaminated soil material, approximately 150 ml of distilled water (free of carbon dioxide) was added to reach the maximum water-holding capacity.

Heavy metal extraction and analysis

After the 30-day incubation period, soil samples were collected from each container. Heavy metal concentrations were determined using single metal extraction methods with DTPA + $CaCl_2$ + TEA (pH 7.3) and 1M NH_4NO_3. The extracted metals were then analyzed to assess the effectiveness of each additive in reducing their mobility.

Statistical analysis

The values obtained through the analysis of soil samples collected from the incubation experiment were processed using several specific methods of mathematical statistics. The first method is analysis of variance (ANOVA), a technique for decomposing the total variance into at least two components that reflect the sources of variation induced in the experiment. By estimating the dispersion of a random variable (soil characteristic in our case), considering the influence of a factor and then removing its influence, comparing the two dispersions provides information regarding the effects of the factor on the studied random variable. If the first dispersion is greater, the factor exerts an influence (Iosifescu et al., 1985).

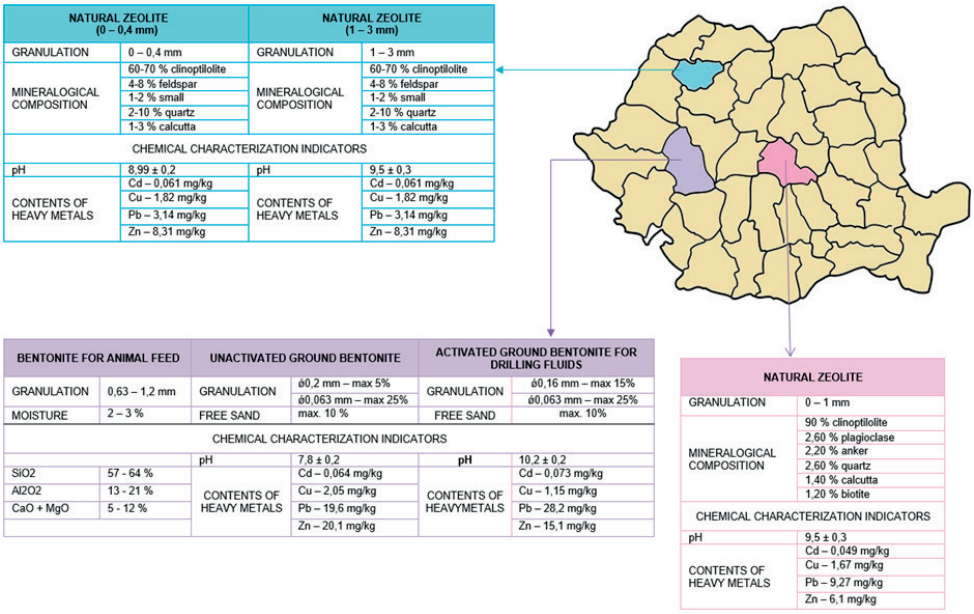


Figure 1. Characterization of inorganic additives of the bentonite and zeolite type used in the case study

RESULTS AND DISCUSSIONS

This study focuses on two primary outcomes from the application of the additives: changes in soil pH and the reduction of heavy metal mobility. The goal is to limit the toxicity of heavy metals and their potential transfer to plants.

Incubation tests using a 3% additive dosage revealed that activated bentonite for drilling fluids ($B_{acff}GS$) from Gurasada caused the most significant increase in soil pH, raising it from 4.59 in the control group to 5.40. This increase is crucial because soil pH significantly influences the mobility of heavy metals. As the soil pH becomes more neutral, the availability of heavy metals to plants decreases. In contrast, bentonite for animal feed ($B_{ha}GS$) had a minimal effect, increasing the pH by only 0.24 units (Figure 2).

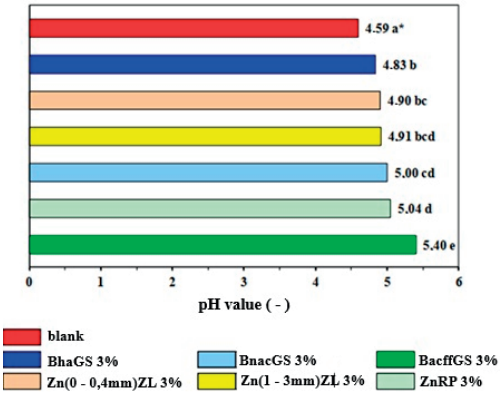
As a result of a study conducted by Vrînceanu et al., 2019, investigating the effectiveness of amendments such as activated bentonite, dolomite, natural zeolites, and manure in limiting the infiltration of heavy metals into the food chain and, implicitly, reducing health risks, an experimental field was established to examine the effects of these amendments on

soils contaminated with heavy metals. The study demonstrated that their application could be an effective solution for reducing the transfer of heavy metals from soil to plants. These amendments have the ability to modify the physicochemical properties of the soil, reducing the bioavailability of heavy metals and their accumulation in plants. The main experimental results showed an increase in soil pH from 5.18 in the control sample to 7.16 in the case of activated bentonite application, as well as a significant decrease in the available concentrations of cadmium, lead, and zinc. The effectiveness of the amendments in reducing the availability of heavy metals followed this order: control > natural zeolite > manure > bentonite > dolomite. At the same time, Soca & Daza-Torres (2016) also state that after the application of zeolite, both the calcium, potassium, and magnesium content, as well as the pH value, increased significantly. An increase in pH value after the application of zeolites in the soil was also recorded by Filcheva & Tsadilas (2002), who conducted a study on the evaluation of the effect of clinoptilolite and compost.

In this case, the effectiveness of the additives in immobilizing heavy metals varied depending on the type of metal, being assessed through extraction with 1M NH_4NO_3.

Analyzing the specialized literature (Misaelides, 2011; Ören & Kaya, 2006), natural zeolites and bentonite have proven to be promising materials for immobilizing heavy metals in contaminated soils due to their unique adsorption and ion exchange properties. O'Connor et al. (2018), suggests that using these natural materials offers significant advantages due to their low cost, availability, and ability to immobilize heavy metals directly in the soil.

In a study conducted by Chen et al. (2000), regarding the evaluation of the effect of various chemical treatments applied to soils (a clayey soil and a sandy soil) contaminated with cadmium (Cd) and lead (Pb), cultivated with wheat, the researchers observed that the accumulation of cadmium and lead in wheat was reduced with the application of calcium carbonate, zeolite, and manganese oxide. Moreover, they found that chemical remediation methods using different treatments could significantly reduce the absorption of cadmium and lead to wheat shoots. The exchangeable (or available) forms of these elements in both cadmium and lead-contaminated soils could be transformed into unavailable forms after modification with 1% manganese oxide or zeolite.



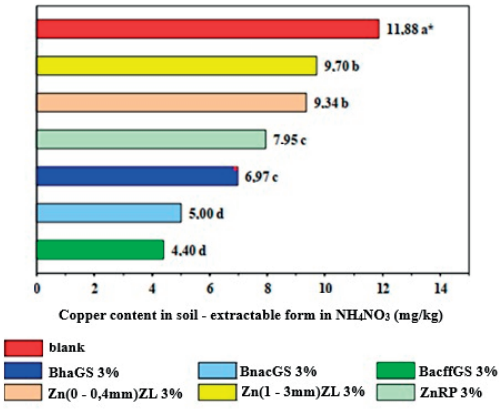
The values followed by the same letter (a, b, ...) do not differ significantly from each other (Tukey's multiple comparison method – significance threshold α=0.05)

Figure 2. Effects of using bentonite and natural zeolite on the pH value of a soil material contaminated with heavy metals

These materials can neutralize soil acidity, reducing the mobility of heavy metals and transforming them into forms that are less accessible to plants. Zeolites and bentonites can also serve as secondary nutrient sources, improving the overall quality of the soil and promoting vegetation regeneration (Bolan et al., 2014). A recent study (Liu et al., 2022) highlights the advantages of combining bentonite and zeolites in the remediation of contaminated soils, suggesting increased efficiency due to the complementarity of their physicochemical properties.

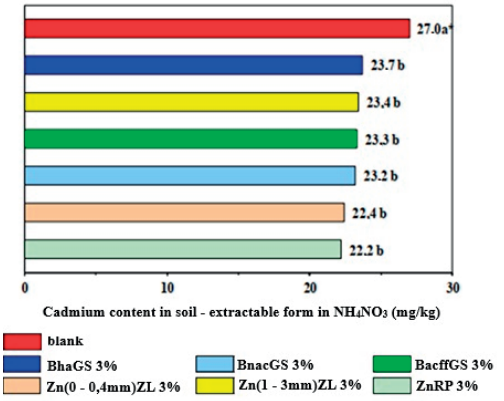
- *Copper*: activated bentonite ($B_{acff}GS$) and natural zeolite from Zalău ($Zn_{(0-0.4)}ZL$ and $Zn_{(1-3)}ZL$) were most effective in reducing copper mobility, with statistically significant decreases of up to 4.91 mg/kg. Natural zeolite from Rupea (ZnRP) also reduced copper mobility, but to a lesser extent, with decreases ranging from 2.18 to 2.56 mg/kg (Figure 3). The higher efficiency of activated bentonite in immobilizing copper could be attributed to its larger surface area and greater cation exchange capacity compared to the natural zeolites. The

activation process may further enhance the adsorption properties of bentonite by increasing its porosity and surface area. The natural zeolites from Zalău, with their smaller particle size, may offer more adsorption sites compared to the zeolite from Rupea, leading to greater reductions in copper mobility.



The values followed by the same letter (a, b, ...) do not differ significantly from each other (Tukey's multiple comparison method – significance threshold α=0.05)

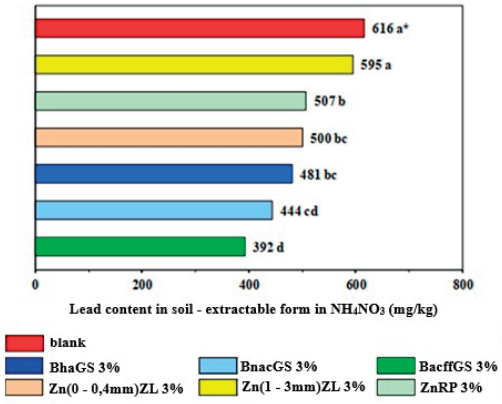
Figure 3. Effects of using bentonite and natural zeolite on the extractable copper content in ammonium nitrate of a soil material contaminated with heavy metals



The values followed by the same letter (a, b, ...) do not differ significantly from each other (Tukey's multiple comparison method – significance threshold α=0.05)

Figure 4. Effects of using bentonite and natural zeolite on the extractable cadmium content in ammonium nitrate of a soil material contaminated with heavy metal

- *Cadmium*: all six additives reduced the mobile concentration of cadmium, but the differences between the additives were not statistically significant (Figure 4). This suggests that the mechanisms responsible for cadmium immobilization may be less influenced by the specific properties of the additives. Possible mechanisms include precipitation of cadmium as insoluble hydroxides or carbonates due to the increase in soil pH, or adsorption onto the surfaces of the additives. The degree of cadmium immobilization at a 3% dose was 17.8% for ZnRP, 17.0% for $Zn_{(0\text{-}0.4mm)}ZL$, 14.0% for $B_{nac}GS$, 13.7% for $B_{acff}GS$, 13.3% for $Zn_{(1\text{-}3mm)}ZL$, and 12.2% for $B_{ha}GS$. As a result of a study conducted by Chen et al. (2000), regarding the evaluation of the effect of different chemical treatments applied to two soils contaminated with cadmium and lead (a clay soil and a sandy soil) cultivated with wheat, they observed that the accumulation of cadmium and lead was significantly reduced with the application of zeolite, calcium carbonate, and manganese oxide in the soil,



The values followed by the same letter (a, b, ...) do not differ significantly from each other (Tukey's multiple comparison method – significance threshold α=0.05)

Figure 5. Effects of using bentonite and natural zeolite on the extractable lead content in ammonium nitrate of a soil material contaminated with heavy metals

- *Lead*: activated bentonite for drilling fluids ($B_{acff}GS$) proved to be the most effective additive in reducing mobile lead concentrations, achieving a significant decrease of 224 mg/kg compared to the control. This reduction surpassed the most effective zeolite by 108 mg/kg. In contrast, natural zeolite from Zalău ($Zn_{(1\text{-}3)}ZL$), with a particle size of 1-3 mm, did not yield statistically significant reductions in lead mobility (Figure 5). This superior performance of bentonite in immobilizing lead aligns with previous studies that have highlighted the strong affinity of

bentonite for lead and its ability to form stable complexes with this metal. The lower effectiveness of the zeolite, particularly the $Zn_{(1-3)}ZL$, could be attributed to its larger particle size, which may limit its surface area and accessibility to lead ions.

- *Zinc*: similar to lead, activated bentonite ($B_{acff}GS$) demonstrated the most substantial reduction in mobile zinc concentration, with a decrease of 409 mg/kg. While zeolite from Rupea (ZnRP) also reduced zinc mobility, its effectiveness was lower, achieving a reduction of 319 mg/kg (Figure 6). This trend suggests that bentonite may be a more suitable additive for immobilizing both lead and zinc in contaminated soils. The higher efficiency of activated bentonite could be attributed to its greater surface area and cation exchange capacity compared to natural zeolites. Moreover, the activation process may further enhance the adsorption properties of bentonite. The lowest degree of immobilization (18.2%) was observed in soils treated with non-activated bentonite from Gurasada ($B_{nac}GS$).

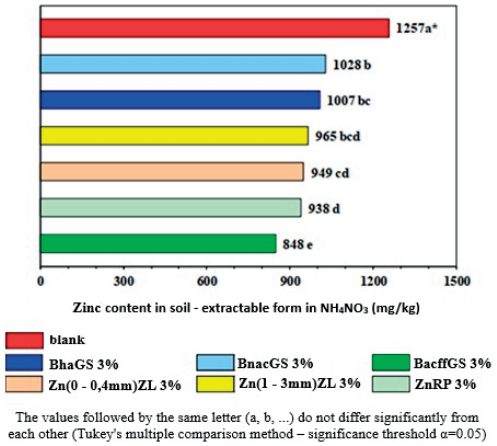


Figure 6. Effects of using bentonite and natural zeolite on the extractable zinc content in ammonium nitrate of a soil material contaminated with heavy metals

CONCLUSIONS

Research has highlighted several important aspects regarding the use of bentonites and natural zeolites for immobilizing metals in contaminated soils. The use of these materials is one of the least expensive and most environmentally friendly practices for reducing the negative effects of soil contamination with heavy metals. By incorporating these materials, which are characterized by a strongly alkaline reaction and high ion-exchange capacity, the aim is to reduce the mobility of heavy metals to limit their transfer from soil to other environmental compartments.

From the incubation tests conducted with 6 indigenous additives (3 bentonites and 3 zeolites) from different sources (Gurasada, Zalău, and Rupea) on a soil contaminated with heavy metals (collected from Copșa Mică), the following observations were made:

pH modification: the application of all tested additives resulted in statistically significant modifications of the soil pH. The most efficient additives in increasing soil pH were activatedbentonite for drilling fluids from Gurasada ($B_{acff}GS$) and natural zeolite from Rupea (ZnRP). At a 3% dose, the pH increase compared to the control was 17.6% ($B_{acff}GS$) and 9.8% (ZnRP).

Copper immobilization: the most efficient additives for copper immobilization were activated bentonite for drilling fluids from Gurasada ($B_{acff}GS$) and non-activated bentonite from Gurasada ($B_{nac}GS$). The incorporation of natural zeolite also led to significant changes in copper immobilization, with the most effective zeolite being the natural zeolite from Rupea.

Cadmium immobilization: all six additives produced statistically significant reductions in cadmium concentrations compared to the control, but there were no significant differences between the additives themselves.

Lead immobilization: the most efficient additives for lead immobilization were activated bentonite for drilling fluids from Gurasada ($B_{acff}GS$) and non-activated bentonite from Gurasada ($B_{nac}GS$). The lowest degree of lead immobilization was observed in soils treated with natural zeolite from Zalău, 1-3mm fraction ($Zn_{(1-3mm)}ZL$). In fact, zeolites were found to be less effective in immobilizing exchangeable lead compared to bentonites.

Zinc immobilization: the most efficient additives for zinc immobilization were activated bentonite for drilling fluids from Gurasada ($B_{acff}GS$) and natural zeolite from Rupea (ZnRP).

Comparison between Bentonites and Zeolites: Bentonites were more efficient in the

immobilization of Cu, Zn, and Pb compared to zeolites. For cadmium immobilization, however, no significant differences were found between the additives studied.

Based on these conclusions, the use of these additives for the immobilization of heavy metals in contaminated soils is recommended, with the note that the 3% doses applied were effective for certain additives depending on the metal studied. The issue of using bentonite or zeolite materials to reduce the negative effects of heavy metal pollution in soils remains an open topic due to its complexity. These materials are inexpensive and environmentally friendly, but soil is a dynamic medium that undergoes continuous changes, which may affect the processes in which metals participate, diminishing the effects of immobilization treatments over time. Therefore, long-term monitoring of contaminated soils treated with these additives is necessary, with treatments being repeated if the effect on metal mobility decreases.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

THE FUNCTIONAL MICROBIAL PROFILE IN HEAVY METAL CONTAMINATED SOILS AFTER ONE AND A HALF YEAR OF BIOREMEDIATION

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Abstract

Bioremediation is a sustainable form of improving the health of soils in heavy metal contaminated urban areas. For the research were selected 5 historically polluted sites from Baia Mare city that were phytoremediated for a period of one and a half year. In all sites, microbial functional profile was analysed in Biolog EcoPlates, a method that enables the detection of microbial heterotrophic communities and their activity in relation to a set of standardized substrates. After a year and a half of bioremediation, the basal activity showed small differences between the microbial functional activity in all 5 analyzed sites. Two sites presented the highest sum of functional activities, with more than 30 units recorded in each. The minimum sum of activity recorded was below 20 units. The same site recorded the highest diversity of the total functional microbiome. The presence of heavy metals is visible in the activation of different functional groups from the total microbial community present in these soils.

Key words*: bioremediation, microbial activity, functional microbiome.*

INTRODUCTION

Soil pollution is a global problem that has already attracted the attention of researchers from various fields, but especially those from the agricultural field, because soil is the main means of agricultural production, which is both source of food for humans and animal feed, and its quality directly influences the quality of life (Ghazaryan et al., 2024), having a significant impact on human health.

Considering the diversity of soil pollutants, a unitary classification using a single criterion could not be achieved, and therefore the sources of contamination and their effects on soil quality, as well as bioremediation methods, must be evaluated separately (Borozan et al., 2021). Most soil pollutants are residues of human activities - biological, domestic and industrial waste, improperly stored waste or manure, which affect the entire soil microbiome and its stability (Briffa et al., 2020; Singha and Chatterjee, 2022). Currently there is a high concern about heavy metal soil pollution, due to their high toxicity, resistance to biodegradation and their ability to accumulate in the long term (Fajardo et al., 2019). For the remediation of these areas, numerous research have been carried out and promising ecological remediation technologies have been developed, which propose the use of plants for the elimination of environmental pollutants (Garbisu et al., 2001).

The current presence of HM into the environment can be associated with the development of mining, metallurgy, industrialization and urbanization, which represent multiple pathways for their accumulation in soils (Ali et al., 2013; Mishra et al., 2023). During the industrial period, in Romania (Baia Mare, Copșa Mică, Zlatna) as in other parts of the world, different levels of soil pollution with heavy metals were recorded in multiple industrial perimeters (Constantinescu, 2008; Haghighizadeh et al., 2024).

Soil microbial community plays an essential role in various fields of human activity, such as agriculture, industry, biotechnology, and health (Malkawi et al., 2024). All biogeochemical cycles that support life on the planet depend on the activity of microorganisms (Brusseau, 2019; Haney et al., 2008). Researchers' interest

in soil microbial imbalances is increasing in the context of soil degradation caused by heavy metals (HM) pollution from anthropogenic activities (Campillo-Cora et al., 2025).

The presence of heavy metals consistently affects both bacterial biomass and its activity (Liu et al., 2020). The heavy metals not only impact soil fertility, but also interfere with bacterial communities, leading to a decrease in biodiversity (Pan et al., 2020). Microorganisms are seen as essential factors in maintaining soil fertility (Johns, 2017). Therefore, it is crucial to understand how soil microbial diversity and composition are influenced by different levels of heavy metal contamination (Azarbad et al., 2015).

The context of soil protection and the conservation of microbial resources within this environment is a necessary step toward more resilient ecosystems (Iqbal et al., 2023). The resilience and recovery potential of ecosystems cand be achieved with holistic approaches that stimulate both the microbial communities from soil and the installation of vegetation (Larson et al., 2022; Peddle et al., 2025). Phytoremediation is a modern technique that uses both the plant and their associated microbiome to remove heavy metals from polluted environments with the aim of restoring it to a similar state as the native one (Azubuike et al., 2016; Sarwar et al., 2017; Thijs et al., 2017).

The use of EcoPlate procedure for the analysis of phytoremediation effect on soil microbiome is a viable instrument to detect the metabolic changes of microorganisms in relation to the removal of heavy metals (Liu et al., 2020). The method provides a physiological profile for soil microbiome and enable the detection of the most active functional groups, based on their ability to decompose a standardized set of substrates (Stoian et al., 2022; Urbaniak et al., 2024).

The aim of this article was to assess the kinetics and dynamics of the functional microbiome in the soils of 5 sites from Baia Mare that were historically polluted with heavy metals, after one year and a half of phytoremediation. The EcoPlate method was used to assess the details of functional microbiome profile, the activity and structure as a response to phytoremediation.

MATERIALS AND METHODS

The soil samples were taken in 2022 from 5 historically polluted from Baia Mare, on which a phytoremediation procedure was applied for one and a half year prior to sampling (Pop et al., 2024; https://uia-initiative.eu/en/uia-cities/baia-mare). The five sites (CR - Craica, CT – Colonia Topitorilor, FR-Ferneziu, ROMP-Romplumb and URB-Urb) are located inside the city of Baia Mare (47°39′ N 23°34′ E), located in the northwestern part of Romania. The locations were selected due to the varying levels of soil contamination with heavy metals and the existence of phytoremediation techniques applied.

For the analysis of soil microbiome traits was selected the Biolog EcoPlates technique, due to its ability of functional microbiome detection and the assessment of their activities in relation to a set of standardized substrates (Pop et al., 2024; Stoian et al., 2022). For these analyses, soil samples were diluted to 10^{-4} prior to the incubation in EcoPlates at room temperature. Measurements were taken at 590 nm using a plate reader, for a period of 5 days. Each reading was performed at 24h, until the readings reached a plateau phase with no further increases in readings observed.

The results from EcoPlates were analyzed according to the methodology proposed by Stoian et al., 2022, which separate the values form plate wells in 5 functional guilds - Carbohydrates (CH), Polymers (P), Carboxylic & acetic acids (CX), Amino acids (AA), Amines/amides (AM). These functional guilds are composed from a different number of functional groups based on their chemical similarity. The functional groups used in the analysis were selected based on their significant change between sites: W – Water (basal community, as a control for the entire set of substrates), CH2 - d-Cellobiose, CH5 - d-Xylose, CH7 - d-Mannitol, CX3 - d-Galacturonic acid, CX5 - 4-Hydroxy benzoic acid, CX7 - Itaconic acid, AA6 - Glycyl-l-glutamic acid, AM1 – Phenylethylamine. Along with these parameters, the recorded sum of all microbial activities (Sum) was used to analyze the differences between the 5 sites in terms of the total microbial community, while Average Well Development Color (AWCD)

was used as an average of metabolic activity (Xie et al., 2006). Diversity indices Shannon-Weiner (H) Simpson (S) and Pielou (J) were used to assess the specific functional diversity of soil microbiome in each site (Lan et al., 2019).

The data analysis was performed in RStudio, version 2022.02.3 (R Core Team, 2024), using the "psych" (Revelle, 2019; Corcoz et al., 2022a) and "agricolae" (de Mendiburu, 2020; Corcoz et al., 2022b) packages. Basic statistics were extracted for all the functional groups and guilds, from which means, and their standard errors were used for the detection of specific physiological level. Diversity indices were calculated in the "vegan" package (Oksanen et al., 2022). Least Significant Differences (LSD) test and ANOVA were used to test the entire database for the assessment of significant differences between the 5 sites.

RESULTS AND DISCUSSIONS

In the current context of the rehabilitation of mining regions, environmental legislation and actors involved in this field, phytoremediation is considered a relatively new, low-cost, environmentally friendly and sustainable method. This technique can be applied in affected mining areas to reduce risks and improve the visual impact on the landscape. (Coman et al., 2009).

Certain heavy metals, such as Cu, Zn and Fe, are necessary for the normal development of microorganisms, but become toxic when present in high concentrations. Heavy metals have been shown to influence microbial populations, having negative effects on cell membranes, growth and metabolic activities, leading to decreased soil microbial biomass and diversity. The tolerance of soil microorganisms to heavy metal contamination varies considerably, and the proportion of resistant culturable microorganisms can fluctuate between 10% and nearly 100% (Abdu et al., 2017; Kamal et al., 2010).

After a year and a half of bioremediation, the basal activity showed small differences between the microbial functional activity in all 5 analyzed sites (Table 1). Two sites presented the highest sum of functional activities, with more than 30 units recorded in each. The minimum sum of activity was observed in the Ferneziu site, where this parameter was below 20 units. The same site recorded the highest diversity of the total functional microbiome.

Table 1. Dimension and diversity of site-specific functional microbiome after bioremediation

Sites	Water	Sum	AWCD	H	S	J
CR	0.07±0.01a	25.39±4.15ab	0.75±0.13ab	2.82±0.11bc	0.93±0.01ab	0.82±0.03bc
CT	0.05±0.03a	29.11±1.24ab	0.89±0.03ab	2.99±0.05ab	0.94±0.00a	0.88±0.01ab
FR	0.07±0.01a	19.18±2.71b	0.55±0.09b	2.72±0.05c	0.91±0.01b	0.79±0.02c
ROMP	0.08±0.00a	34.87±2.19a	1.05±0.07a	3.06±0.01a	0.95±0.00a	89±0.00a
URB	0.06±0.01a	31.77±5.98a	0.96±0.19a	2.98±0.10ab	0.94±0.01a	0.87±0.03ab
F test	0.65	2.76	2.87	3.44	4.04	3.77
p.val	0.640	0.088	0.081	0.052	0.033	0.040

Note: means ± s.e. followed by different letters present significant differences according to LSD ($p<0.05$). Legend: sites: Craica (CR), Colonia Topitorilor (CT), Ferneziu (FR), Urbis (URB), Romplumb (ROMP). Diversity indices: H – Shannon, S – Simpson, J – Pielou.

In the water group (basal community), the highest level of microbial activity was observed in the ROMP site with a value of 0.08, and the lowest level in the same group was recorded with a value of 0.05 in CT (Table 1). Compared to these values, CR, FR and URB sites do not show significant differences.

The sum of microbial activities shows significant differences between analysed sites. The lowest functional activity was recorded in FR site, with less than 20 absorbance units Compared to this value, CT, ROMP and URB sites showed an activity with 55-65% higher. CR site present an average activity, with 6 units higher than FR and almost 10 units compared to ROMP. A significant difference was identified in ROMP site which showed a microbial activity with 8.9 units higher than FR. After comparing the results from the CR, CT and URB sites, there are no insignificant differences. Following the analyzed values of the AWCD variable, significant differences between the sites were recorded. The highest average activity was identified in ROMP,

followed by URB. On the other hand, the lowest value was identified in FR, which presents significant differences from the maximum value, but also from the CR and CT sites. An increase in microbial activity was observed by comparing the values of the sites analyzed. In the ROMP site, which has the highest activity, and FR, which registers the lowest analyzed value. There are no significant differences between CT and URB locations. CR shows 1-unit lower activity than ROMP.

The elimination of heavy metals from the environment represents a major challenge because their decomposition, as in the case of other pollutants, cannot be achieved by biological or chemical methods (Sharma et al., 2023).

The highest diversity (H index) value was recorded in ROMP. Compared to this value, CT and URB locations do not show significant differences (Table 1). The site with the lowest value recorded in FR shows a significant difference compared to ROMP. Insignificant difference of the activities shows the results in CR, compared to CT and URB.

Heavy metals are known for their ability to reduce or inhibit soil enzymatic activity, disrupt carbon, nitrogen, and organic matter transformation processes, and decrease both biodiversity and soil microbial biomass (Giller et al., 2009). As a result, this may favor the emergence of certain microorganisms resistant to heavy metals in the soil (Giller et al., 2009). Soils polluted with heavy metals restrict plant growth due to their toxicity. In addition, heavy metal toxicity influences the size of microbial populations, their diversity, activity and genetic structure (Ayangbenro & Babalola, 2017).

In the analyzed group of polymers, a significant difference is registered in the ROMP site, which presented an activity 1 unit higher than FR, with the lowest value (Table 2). Compared to ROMP, URB does not show significant differences. Similarly, the values in CR and CT show no significant differences.

In terms of CH guild activity, a significant difference is observed between ROMP site, which presents the highest analyzed value, with 50% compared to FR, which has the lowest activity level and respectively 4 units compared to the CR site. The CT and URB sites are significantly different from each other by 1 unit.

The highest recorded value for CX is presented in the ROMP site, and the lowest activity in FR There is a significant difference between the two locations of 60%. After comparing the results between CT and URB, we notice that there are no significant differences, but it shows a difference of 1 unit higher than CR.

Between the sites analyzed for AA guild, the highest microbial activity was recorded in URB, with a value of 7.81, and the lowest value of 3.82 in FR. There was a significant difference between the two locations. The CR site shows a difference of 2 units from the FR. CT and ROMP values show no significant differences.

In the analyzed group AM, significant difference of 1 unit exists between the maximum value of 1.71 in URB and the minimum value of 0.96 in CT. Compared to the maximum value analyzed, the CR, FR and ROMP sites do not show significant differences.

Table 2. Site-specific effect of bioremediation on functional guilds activities

Sites	P	CH	CX	AA	AM
CR	3.78±1.05a	6.37±0.23bc	8.73±1.99ab	5.21±1.45ab	1.31±0.29a
CT	3.40±0.20a	8.92±0.37ab	9.30±1.54ab	6.54±0.70a	0.96±0.58a
FR	3.32±0.87a	5.09±0.71c	5.40±1.31b	3.82±0.85b	1.55±0.60a
ROMP	4.35±0.65a	10.07±0.98a	11.86±1.68a	6.90±0.08a	1.68±0.16a
URB	4.01±1.07a	9.06±1.31a	9.17±2.61ab	7.81±0.38a	1.71±0.80a
F test	0.27	6.46	1.5	3.54	0.34
p.val	0.893	0.008	0.273	0.048	0.846

Note: means ± s.e. followed by different letters present significant differences according to LSD ($p<0.05$). Legend: sites: Craica (CR), Colonia Topitorilor (CT), Ferneziu (FR), Urbis (URB), Romplumb (ROMP).

For CH2 functional group the highest activity was recorded in the CT site, with a value of 1.56, and the lowest activity in CR, presenting a significant difference (Table 3.). The results

between FR and CR show no significant differences. There was also no significant difference in activity between ROMP and URB sites. In the CH5 functional group, there was a significant difference between the CT site with the highest value of 0.98 and the CR site with the lowest value of 0.07. FR and URB sites show no significant differences between them. ROMP showed a 0.91 increase in activity over CR.

After analyzing the CH7 group, the ROMP site shows an activity 2 units higher than FR resulting in significant differences between them. URB with the value of 2.17, does not show significant differences compared to ROMP. CR and CT sites show higher activity than FR.

The highest functional activity of CX3 was recorded in ROMP with a value of 2.25 and the lowest activity was recorded in FR with a value of 0.15 (Table 3). The URB and CR sites do not show significant differences between them. Functional activity in the CT site was 1 unit higher than in FR. A significant difference of 1 unit was observed between ROMP and FR sites for CX5 group. CR and URB sites show no significant differences. Compared to these values, a decrease in microbial activity was observed in CT.

The analysis of CX7 functional group values shows significant differences. The highest value was recorded in ROMP with more than 91% compared to CT, which has the lowest activity. FR and URB sites show no significant differences between them. Compared to these values, a decrease in CR activity was observed.

The highest activity of AA6 functional microbiome was recorded in the URB site with a value of 0.81, and the lowest activity was recorded in the CR site with a value of 0.14. Compared to these values, CR shows 64% lower activity than CT. There are no significant differences between the values of the FR and ROMP sites.

Table 3. Differences between the most significant activities of functional groups after bioremediation

Sites	CH2	CH5	CH7	CX3	CX5	CX7	AA6
CR	0.82±0.11b	0.07±0.01b	1.13±0.62ab	1.45±0.70ab	1.72±0.24a	1.18±0.56ab	0.14±0.07b
CT	1.56±0.09a	0.98±0.14a	1.47±0.45ab	1.99±0.06a	1.02±0.56ab	0.07±0.00b	0.78±0.07a
FR	0.83±0.06b	0.43±0.15ab	0.65±0.58b	0.15±0.08b	0.23±0.17b	1.85±0.14a	0.26±0.16ab
ROMP	1.05±0.08b	0.96±0.41a	2.21±0.13a	2.25±0.05a	1.73±0.22a	1.92±0.18a	0.20±0.05b
URB	1.02±0.13b	0.31±0.24ab	2.17±0.06a	1.52±0.72ab	1.60±0.51a	1.38±0.63a	0.81±0.34a
F test	9.78	3.07	2.38	3.2	2.96	3.69	3.44
p.val	0.002	0.068	0.121	0.062	0.075	0.043	0.051

Note: means ± s.e. followed by different letters present significant differences according to LSD ($p<0.05$). Legend: sites: Craica (CR), Colonia Topitorilor (CT), Ferneziu (FR), Urbis (URB), Romplumb (ROMP).

CONCLUSIONS

The presence of heavy metals was visible in the activation of different functional groups from the total microbial community present in these soils. The basal community showed reduced activities within all analysed sites but performed well in specific functional groups and guilds.

The maximum sum of activities was recorded in Romplumb and Urbis sites, with more than 31 absorbance units each.

Carbohydrates and Carboxylic & acetic acids functional guilds showed the highest metabolic activities in Colonia Topitorilor, Romplumb and Urbis sites.

Amino acids guild showed the lowest activity in Ferneziu site, while Amines/amides guild had an activity bellow 1 unit in Colonia Topitorilor site.

The most significant activities of functional groups were recorded in Romplumb site (CH7 and CX3).

Ferneziu site presented the lowest activities for CH2, CH5, CH7, CX3 and CX5 compared to the other 4 sites analysed.

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*** https://uia-initiative.eu/en/uia-cities/baia-mare

Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

THE BEHAVIOR OF WINTER WHEAT IN THE CONSERVATIVE TILLAGE SYSTEM UNDER THE CONDITIONS AT A.R.D.S. PITEȘTI - ALBOTA

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Abstract

In the conservation agriculture, minimum tillage and direct sowing have been widely spread lately in the wheat crop. The paper presents the experimental results obtained in the agricultural year 2023-2024 regarding the effect of pedoameliorative and basic soil works - conventional and conservative system (direct sowing) on the wheat crop. The research was carried out in the experimental field of the ARDS Pitesti-Albota. The yield varied according to the pedoameliorative works (scarified soil, nonscarified soil), the depth of the basic soil works, but also the climatic conditions recorded during the research period. In the experimental year 2024, the average wheat yield recorded a value of 5683 kg/ha in the scarified soil variant and 5399 kg/ha in the nonscarified soil variant, with a difference of 284 kg/ha in favor of the scarified soil variants. From the data obtained in the reserch area of ARDS Pitesti Albota in the conditions of the typical luvosol, the conservation system was favorable to the wheat crop.

Key words*: conservative system, grain yield, luvosol, soil works, wheat.*

INTRODUCTION

Agriculture is a vital human activity, providing food, clothing and shelter. It has been shown that
because for every 1% rise in agricultural output, there is a 0.6-1.2% drop in the number of poor households worldwide (Thirtle et al., 2001).
The main concern of the Romanian rural community is agricultural yield, which includes the cultivation of winter cereals, especially wheat (Lipianu et al., 2023).
Wheat (*Triticum aestivum* L.), the most significant grain and the plant that covers the most land worldwide, is essential to human nutrition (Erenstein et al., 2022).
High yielding wheat varieties capacity must be resistant to important climatic risk factors in Romania, must be resistant to major foliar diseases and allow the growth of quality crops.
As per the study published by the United States Department of Agriculture, wheat is the most significant cereal crop in the European Union, with an expected global yield of 789 million tonnes in the 2023/2024 agricultural year. In 2022, wheat, considered one of the most common foods in the world, was sown on 2.15 million hectares in Romania, according to the National Institute of Statistics. In Romania, the total amount of wheat harvested in 2022 was 8.559 million tonnes, despite estimates that the world wheat harvest was the second largest of all time (http://www.fao.org/faostat).
The practice of conventional tillage can negatively affect soil yield in the long term, leading to erosion and loss of soil organic matter. Conservative tillage, crop rotation, and the retention and utilization of crop residues are methods for achieving sustainable soil management (Hobbs et al., 2008). Research carried out in a variety of climatic circumstances, soil types and crop rotation systems have shown that soils under direct seeding and minimum tillage have have a significantly higher content of organic matter compared to conventionally cultivated soils (Alvarez, 2005).
The success of wheat cultivation, both in terms of productivity and quantity, depends on the pedoclimatic conditions that are affected by the applied technology (De Vita et al., 2007a;

Warechowska, 2009; Ceclan et al., 2015; Zain et al., 2015). In addition, a long-term study showed that, compared to agricultural practices, the variation in climatic conditions explained a higher proportion of the variability in wheat crop yield. This highlights the importance of considering this vital parameter in predicting crop productivity (de Cárcer et al., 2019).

It is difficult to predict the reaction of wheat crop to the tillage system, as yields are affected by a variety of factors (Liliane et al., 2020), including soil characteristics, microclimate and the association of different practices (soil preparation, sowing dates), equipment used, crop rotation, hybrids used, fertilization and weed control (Moraru et al., 2012).

Wheat quality is not solely determined by its technological qualities; customers are becoming more conscious of and worried about the grain's nutritional value (fiber, minerals, macro- and micronutrients, vitamins), as well as any potential health effects. For instance, cooperation between wheat protein geneticists and allergy specialists will be necessary for study on a number of diseases linked to the use of gluten-based goods (Igrejas & Branlard, 2020).

Conventional agricultural yield systems use agrochemicals with negative environmental impacts. These inputs are not exploited in organic systems, instead biological inputs are applied. Even if the effects on the environment are less severe than those of traditional farms, depending on the production area, organic wheat yields are disproportionately low (Smith et al., 2019).

Wheat yield uses material and energy inputs, including fertilizer and fuel, for various operations (Vinci et al., 2022). The huge amount of agrochemicals and fuels associated with global wheat yield has had serious environmental implications since the Industrial Revolution and, more recently, the Green Revolution (Acevedo et al., 2018).

The technology of cultivating winter wheat in a conservative system (the minimal tillage variant and the direct sowing variant in stubble) is practiced on increasingly large areas and aims to reduce energy consumption, conserve soil and water, organize an adequate crop rotation, protect plants with environmentally friendly substances, and reduce soil erosion and compaction.

The conservative system (no-tillage) has the advantage of better water conservation in the soil, which can be gradually used by plants during drought periods. The economic efficiency of the no-tillage tillage system results mainly from the reduced fuel consumption (approximately 50%) and the reduced number of soil works, which determines a reduction in total technological costs (Cheţan et al., 2017). Not only the tillage system, but also the type of soil and the agricultural machinery used have a major effect on cereal yields (Ferrández-Pastor et al., 2018).

This paper describes the effect of tillage systems on crop establishment, quality indices and yield of wheat crop.

MATERIALS AND METHODS

The paper contains experimental results obtained in the 2023-2024 agricultural year in the cultivation of winter wheat, by practicing a technology based on soil improvement (scarified, nonscarified) and basic soil works, in a classical system (deep plowing, normal plowing) and conservative (discultivation, direct sowing in stubble).

The three year crop rotation (corn, peas, and wheat) took place at the experimental field of A.R.D.S. Piteşti in 2023/2024 season. The study was conducted on a typical luvosol soil type, which has a clay texture (27.2% clay content in the 0-20 cm layer), poorly supplied with nitrogen and phosphorus (Nt = 0.130% mg/, PAL = 31 mg/kg), moderately supplied with potassium (KAL = 83 mg/kg) with a humus content in the arable horizon of 2.1% and pH = 5.3.

The experimental design method used was that of subdivided plots arranged according to the completely randomized block method in four repetitions.

As part of the experiment, variants with four graduations were established (deep plowing, normal plowing, discing and direct sowing), on two agro-funds: scarified and nonscarified.

Plot dimensions were 560 m^2 (5.6 x 100 m).

In the autumn of 2021, scarification was only done at the experiment's establishment, at a depth of 40-50 cm, the wheat crop benefiting from the effects of loosening in the third year of cultivation.

Loosening is the right solution for working the soil after repeated plowing, breaking the formed hardpan, achieving deep loosening, which contributes to improving the aerohydric regime and increases the amount of water stored in the soil.

Scarification is not a long-term solution, because soils easily recomplicate and it is necessary to repeat it, and over time the intensity of compaction and recompaction increases. This is why these soil improvement works also require other measures to prevent compaction, which should include long-term rotations with improving plants, organic fertilization and a rational soil tillage system.

In the conventional (classic) system, sowing was carried out on 26.10.2023, with the SUP-31 seeder, at a sowing depth of 4cm-5cm. The wheat variety used was Ursita. The sowing density was 550 germinating seeds/m², corresponding to a quantity of 281 kg/ha of seed.

In the unconventional (no till) system sown directly into stubble, sowing was also carried out on 26.10.2023, with the Mzuri Pro-Til 3T Select seeder from ETU-Farm under the same technological conditions as in the conventional system.

The fertilization formula used was N_{128} P_{92} K_0 s.a. kg/ha. Basic fertilization was carried out before sowing where complex fertilizers of the $N_{18}P_{46}K_0$ type were applied, in a quantity of 200 kg/ha of commercial product. In the spring, when the plants resumed vegetation, the nitrogen dose was completed by applying the commercial product urea 200 kg/ha.

In 2024, a treatment with the herbicide Floramix at a dosage of 260 g/ha plus 0.6 l/ha Dassoil adjuvant was conducted to manage weeds. The fungicide Falcon Pro was sprayed at a rate of 0.6 l/ha to combat ear and foliar diseases.

The wheat crop was harvested on 1.07.2024.

The results obtained were processed and statistically analyzed using the Polifact program.

The constantly changing climatic conditions have negative influences on the physiological mechanisms of cereal development, which are disrupted in particular by the prolonged droughts that are increasingly frequent in our country (Berca et al., 2023).

The research station is located in an area with a temperate continental climate, with a multi-annual average temperature of the researched period over the last 10 years of 10.1°C.

The sum of multiannual rainfalls for the period is 609.4 mm. Its distribution is completely uneven, both during the year and from one year to another.

During September - July 2023-2024, temperatures and rainfalls were monitored and recorded using the weather station within the resort to track the influence of environmental factors on plant growth and development (Figures 1 and 2).

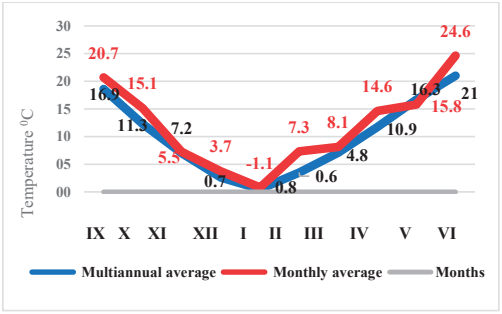


Figure 1. Temperature evolution between September 2023 to June 2024

Temperatures recorded between September and June (the growing season of wheat) recorded a positive average deviation of 1.7°C compared to the multiannual average.

The climate data for the 10 months characterize the year 2023-2024 as warm and dry, with increased temperatures with positive deviations from the multiannual average except for May which recorded a negative deviation of -0.9°C.

In the agriculture year 2023-2024, the average annual temperature recorded was 11.8°C, exceeding the multiannual average temperature of 10.1°C by 1.7°C (Figure 1).

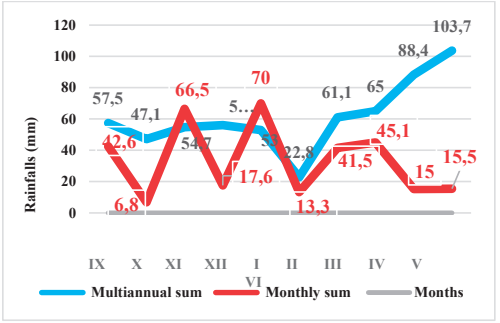


Figure 2. In 2023-2024 total monthly rainfall during the cropping season (September to June) at ARDS Pitesti

The climatic data of the researched period characterize the agricultural year 2023-2024 as dry, with low rainfalls with negative deviations from the multiannual amount, with the exception of the rainfalls in January which recorded a positive deviation of 17 mm compared to the multiannual amount of 53 mm. The monthly rainfalls sum for the period recorded a value of 334.2 mm, registering a deficit of -275.2 mm, compared to the multiannual sum of 609.4 mm.
Under these conditions, with temperatures with a positive deviation compared to the annual average and rainfalls with a negative deviation compared to the multiannual average, wheat sown in the optimal period emerged with a very long delay, staggered, with complete emergence occurring in the second decade of December, which no longer allowed the twinning period to be completed in the autumn.

RESULTS AND DISCUSSIONS

The correct choice and application of tillage systems has a significant effect on grain yield and quality. They can play a decisive role in achieving optimal wheat yields, directly influencing not only yield but also long-term soil health and sustainability (Chetan et al., 2017).

The studied factors (scarified, nonscarified; working depth of basic soil works) as well as the climatic conditions recorded during the research period influenced winter wheat yield.
In the fall season of 2021, scarification only took place at the experiment's establishment, at a depth of 40-50 cm. The wheat crop benefited from the effects of loosening in the third year of cultivation

Table 1. Influence of soil scarification on wheat yield

Factor A	Grain yield (kg/ha)	%	Diff.	Semnif.
A_1 - nonscarified soil	5398.7	100	-	Mt
A_2 - scarified soil	5683.4	105.3	284.6	***
LSD 5%		1.26	68.03	
LSD 1%		2.17	117.18	
LSD 0.1%		5.12	276.68	

As can be seen from Table 1, the average wheat yield on nonscarified soil recorded a value of 5398.7 kg/ha, and on scarified soil 5683.4 kg/ha with a very significant positive difference of 284.6 kg compared to the control variant.
As can be seen from Table 1, the average wheat yield on nonscarified soil recorded a value of 5398.7 kg/ha, and on scarified soil 5683.4 kg/ha with a very significant positive difference of 284.6 kg compared to the control variant.
From the results obtained on the soil (typical luvosol) in the Pitesti high plain area, it is observed that the scarification work performed indicates an increase in wheat yield.

Table 2. Influence of soil tillage on wheat yield

B-Factor	Grain yield kg/ha	%	Diff.	Semnif.
b_1 - deep plowing (control variant)	5898	100	-	Mt
b_2 - normal plowing	5434	92	-464	000
b_3 - disc	5298	89.8	-600	000
b_4 - direct sowing	5534	93.8	-364	00
LSD 5%		3.71	218.9	
LSD 1%		5.08	299.8	
LSD 0.1%		6.92	408.6	

In the conventional (classic) tillage system, deep plowing considered as the control variant, the yield value was 5898 kg/ha. The normal plowing variant recorded a value of 5434 kg/ha, with a very significant negative difference of 464 kg/ha compared to the control.
The average yield in the conservative system with minimal soil work (disc) had a value of 5298 kg/ha, the difference of 600 kg/ha is very significantly negative compared to the control variant.
In the conservative soil tillage system, direct sowing variant, yield recorded a value of 5534 kg/ha, with a distinctly significant negative difference of 364 kg/ha compared to the control variant (Table 2).

Evolution of morphological characters of wheat in the conservative tillage system

Regarding the influence of the two factors, the total biomass production ranged between 12550 kg/ha and 16825 kg/ha. On the nonscarified soil agrofond, the differences were smaller (12550-15475 kg/ha).
From the data of the factor A nonscarified soil it results that the highest increase was in the control variant (deep plowing), then slightly

lower but similar values were recorded in normal plowing and disc. A positive element was observed in the direct sowing variant of wheat where the total biomass yield was 13025 kg/ha, respectively 500 kg/ha more than normal plowing and disc. The explanation is given by the fact that although the achieved density was lower (the nutrition space is larger) the plants had a more vigorous development compared to the normal plowing and disc variants.

Under conditions where the soil was scarified, significant increases were recorded in all four tested variants.

Table 3. The influence of experimental factors on wheat yield elements

Factor A	Factor B	Biomass yield (kg/ha)	%	Ears yield (kg/ha)	%	Grain yield (kg/ha)	%	TGW (g)
A_1 nonscarified soil	**deep plowing control variant**	**15475**	**100**	**8993**	**58.1**	**5627**	**36.4**	**36.9**
	normal plowing	12675	81.9	7543	59.5	5347	42.2	36.8
	disc	12550	81.1	7428	59.2	5144	40.9	36.0
	direct sowing	13025	84.2	7583	58.2	5477	42.0	37.6
A_2 scarified soil	**deep plowing**	16825	108.7	9123	54.2	6169	36.7	37.2
	normal plowing	13725	88.7	7738	56.4	5520	40.2	37.1
	disc	13688	88.4	7635	55.8	5453	39.8	36.9
	direct sowing	15625	101.0	7763	49.7	5590	35.8	38.4
Averange		**14198.5**		**7975.7**	**56.4**	**5541**	**39.3**	**37.1**
LSD 5%		**1093.55**	7.06	**222.93**	2.48	**381.77**	6.78	**0.77**
LSD 1%		**1603.28**	10.36	**313.39**	3.48	**524.37**	9.31	**1.14**
LSD 0.1%		**2698.33**	17.43	**466.16**	5.18	**721.49**	12.82	**2.00**

The highest biomass yield was obtained in the conventional system on deep plowed scarified soil of 16825 kg/ha. In the normal plowing and disc plowing variants the values were close, 13688-13725 kg/ha. In the direct sowing variant the same phenomenon of increase in the amount of biomass (15625 kg/ha) was observed with a significant effect (Table 3).

In terms of percentage, it was found that in the nonscarified variants, biomass yield represented between 81 and 84% of the deep plowed control. In the scarified variants, increases were found over the control (deep plowing and direct sowing over 100%), and in the normal plowed and disced variants, biomass approached 90% of the control value.

Regarding the yield of ears, values between 7428 and 8993 kg/ha were recorded on the nonscarified agricultural land. With the scarification action, the yield of ears was somewhat higher, namely between 7635 and 9123 kg/ha.

In terms of percentage, in the variants on nonscarified soil, the ear yield represented between 82 and 84% of the control. On the scarified soil, the ear production was expressed at higher values, between 7635 and 9123 kg/ha. These constituted 95-96% in the variants with normal plowing, disc plowing, direct sowing, while in the variant with deep plowing, wheat produced 1.4% more than the experimental control.

Grain yield primarily expressed a characteristic of the crop year.

In the nonscarified soil variant, a certain uniformity of the yield level was found, between 5144 and 5627 kg/ha. From a statistical point of view, there are slight significant differences. In the scarified soil variant, differences were found, between 5453 and 5590 kg/ha on the last three variants and over 6169 kg/ha in the deep plowed variant.

From a percentage point of view, the figures follow the grain yield values, namely below 91-97%.

In the case of scarification, the deep plowing variant induced a significant increase, compared to the rest of the variants which were below the control level.

Regarding the absolute mass values of wheat grains, a relative uniformity was found throughout the experiment with one exception. Thus, the TGW had values between 36 and 37.6 g in the first seven variants, including the variant control. The exception was variant eight with direct sowing on scarified soil, where the value obtained was 38.4 g. One of the explanations would be that in this wheat crop

year, the climatic conditions and the scarification work ensured more favorable conditions.

Analyzing the entire experiment in the 2023-2024 agricultural year, it was found that the Ursita wheat variety produced 14199 kg/ha of total biomass. Considering that most winter wheat varieties yield between 14000 and 16000 kg/ha of total biomass, we consider that in this crop year the quantity obtained was satisfactory.

Of the total biomass obtained, the ears represented an average quantity of 7976 kg/ha, which represents a percentage of 56.4%. We also consider in this case that the percentage in which the plant produced ears biomass was high. From a biological point of view, the Ursita variety, having a relatively low to average height, the spike biomass is considered more than satisfactory.

The average grain yield was at an average level of 5541 kg/ha. In percentage terms, this production constituted 39.3% of the total wheat biomass. Given this percentage of grains in the total biomass, it is considered that the Ursita variety can be classified as an intensive variety. Grain yield and the assessed crop's quality indices are both greatly increased by planting winter wheat following peas. Plaza-Bonilla et al. (2017) also showed that prior leguminous crops had a favorable impact on wheat output, as they acquired a better grain yield following a pea (*Pisum sativum* L.) crop than following a sunflower (*Helianthus annuus* L.).

Table 4. Influence of soil tillage on wheat quality indices

Factor A	Factor B	Protein PB (%)	Starch (%)	Gluten (%)	Zeleny index (ml)	MH (kg/hl)
A_1 nonscarified soil	deep plowing control variant	12.4	69.7	24.1	37.9	84.5
	normal plowing	12.1	69.2	22.8	36.5	83.6
	disc	12.0	67.8	24.0	41.5	85.6
	direct sowing	11.4	70.0	21.6	31.7	85.2
Averange		11.9	69.2	23.1	36.9	84.7
A_2 scarified soil	deep plowing	12.9	68.7	25.1	41.3	83.9
	normal plowing	12.9	68.5	25.9	44.0	84.5
	disc	12.7	68.7	25.1	41.5	85.7
	direct sowing	12.4	69.1	24.0	37.3	84.4
Averange		12.7	68.7	25.0	41.0	84.6
Total averange		12.3	68.9	24.1	38.9	84.66
LSD 5%		0.85	1.97	2.69	9.53	1.40
LSD 1%		1.20	2.91	3.96	14.24	2.05
LSD 0.1%		1.85	4.99	6.79	25.22	3.44

To establish the quality indices, the NIR PERTEN analyzer model Inframatic-9500 was used.

Regarding crude protein (PB %) in the nonscarified variants, the values were between 11.4 and 12.4%. From a qualitative point of view, the soil tillage variants, disc and direct sowing, fall into the second category, while the deep plowing and normal plowing variants correspond to the first quality category. By applying the scarification work, the PB % values fell between 12.4 and 12.9%, which corresponds to the first quality category for all variants. The average crude protein value recorded 12.3%, which corresponds to the first quality class, in all soil tillage systems (Table 4). Similar results were obtained by Leonte et al. (2024), who showed that the soil tillage system did not influence the protein, oil and starch content of wheat grains, these quality indices registering close values between the studied variants.

On the nonscarified soil, the starch content was between 67.8 and 70%. Of these, only the disc variant was in the good starch category. The average of the A_1 factor (69.2%) is in the very good category. By scarifying the soil, values between 68.5 and 69.1% were obtained.

After analyzing the results obtained, it is observed that the average starch content in the variants carried out on scarified soil was below the very good category.

At the level of the entire experiment, it was found that the average starch (68.9%) is slightly below the conditions of the very good category.

Wet gluten recorded values between 21.6 and 24.1% on the agrofond nonscarified soil. The highest values were obtained in the deep plowing and disc plowing variants, respectively 24%. The average gluten content in wheat grains in the nonscarified soil variants was 23.1%. By scarification, the gluten content varied between 24% (direct sowing) and 25.9% (deep plowing). The average of the A_2 factor (scarified) was 25.0% (wet gluten). By comparing the average of the two A factors, a difference of 1.9% more gluten in favor of the scarified soil variants is found (Table 4). At the level of the entire experiment, wet gluten had an average of 24.1%, which demonstrated a satisfactory quality classification.

The fourth characteristic studied, namely the Zeleny index, characterizes the possibility of classifying bread wheat according to certain indices.

The four soil works performed without scarification recorded Zeleny indices ranging between 31.7 and 41.5 (ml). The highest value of the Zeleny index was obtained in the conservative system, the minimal-disc works variant, 41.5, and falls into the good category. The average of the A1 factor (unscarified soil) was 36.9, also falling into the good category. Through the scarification work, the wheat recorded Zeleny index values ranging between 37.3 and 44.0 (ml). The highest value was obtained in the normal plowing variant. At the level of the entire experiment, the Zeleny index was 38.9, which places the wheat quality in the Good category. The average of the works based on scarification was 41.0, which means good quality (Table 4). Similar results were also obtained by Bărdaș et al., in a two-year experience (2015-2017) carried out at the ARDS Turda, the protein percentage obtained by them being between 12.1 and 13.8%, gluten between 23.5 and 27.3%, and the Zeleny index between 35 and 48% (Bărdaș et al., 2024).

From the data presented in Table 4 it appears that the highest value of the hectoliter mass MH was recorded in the conservative system, the variant with minimal disk work on scarified soil (85.7 kg/hl). The lowest value of MH (83.6 kg/hl) was obtained in the conventional system, the variant with normal plowing.

The tillage system did not determine significant differences in the average hectoliter mass MH, the values recorded being 84.7 on nonscarified soil and 84.6 on scarified soil.

The soil tillage system applied to the wheat crop influenced both yield and quality indices, falling into the good quality group.

The yield and technological value of wheat grains are genetically determined and depend mainly on the properties of the variety, as well as on agronomic and environmental factors (Horvat et al., 2015)

The correlations of the qualitative indices of wheat production (Ursita) concerned: crude protein (PB%), starch content, gluten, Zeleny index, TGW and MH (Table 5; Figures 3-6).

Regarding grain yield, it had insignificant positive relationships with protein, gluten, Zeleny index and TGW and insignificant negative correlations with starch and MH.

Table 5. Correlations obtained regarding the values of the qualitative indices studied in wheat

	Grain yield (kg/ha)	**Protein (PB %)**	**Starch (%)**	**Gluten (%)**	**Zeleny index (ml)**	**TGW (g)**	**MH (kg/hl)**
Grain yield (kg/ha)	1	0.164	-0.095	0.109	0.045	0.193	-0.298
Protein (PB %)		1	-0.254	**0.793**	**0.641**	0.074	-0.059
Starch (%)			1	**-0.551**	**-0.803**	**0.375**	-0.028
Gluten (%)				1	**0.891**	-0.146	0.141
Zeleny index (ml)					1	-0.282	0.124
TGW (g)						1	-0.248
MH (kg/ha)							1
	DL 5%=0.35		**DL 1%=0.45**		**DL 0.1%=0.55**		

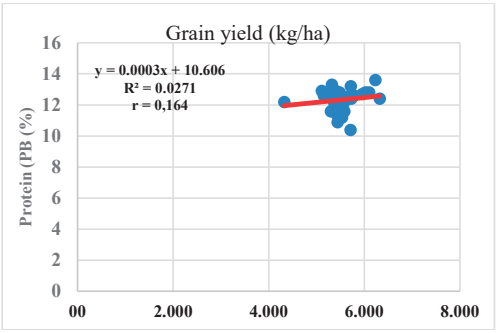


Figure 3. Correlation between crude protein content (PB %) and grain yield (kg/ha)

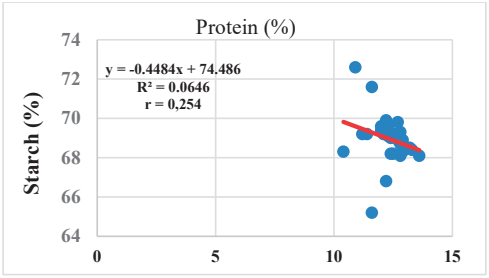


Figure 4. Correlation between starch content and crude protein (%)

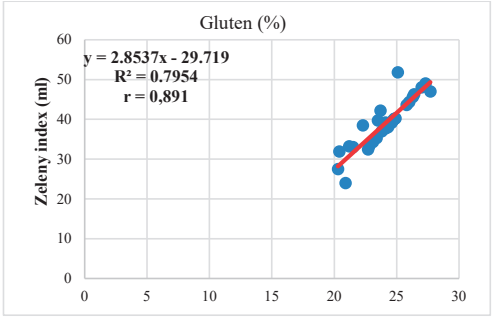


Figure 5. Correlation between Zeleny index (ml) and gluten %

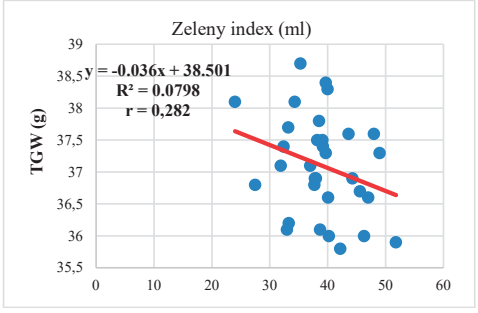


Figure 6. Correlation between TGW (g) and Zeleny index (ml)

Regarding the links (correlations) between the quality analyses performed, different situations were found. From the data obtained, both positively significant and negatively significant situations were found. Regarding the correlations with the grain yield obtained, it is establish that protein and gluten along with TGW demonstrated positive but insignificant links. This situation proved positive in the experiment due to the fact that the variants favored the formation of crude protein (PB%) and additional gluten.

Grain gluten has a very significant positive correlation with the Zeleny index and demonstrates a very favorable direct link in bread production. Another favorable element was found between gluten and MH through the positive correlation coefficient, a necessary element in the valorization of wheat grains.

The protein content of cereals is one of the most important criteria for determining the quality of common wheat (*Triticum aestivum*). One of the major obstacles to the yield of wheat for bread making is the negative correlation between protein content and grain yield (Geyer et al., 2022).

This is backed up by another positive feature, specifically the rise in the Zeleny index, which is advantageous when obtaining bakery goods. However, the development of heavier grains also contributed to the rise in wheat grain output.

CONCLUSIONS

In the research with conservative works (disc and direct sowing) in the winter wheat crop, a specific improvement was generally found in both plant biomass and quality analyses on the two agrofund systems (nonscarified soil-scarified soil).

The experimental results obtained on wheat crops in the 2023-2024 agricultural year, under the conditions at ARDS Pitesti, indicate an increase in wheat yield in scarified variants, thus resulting in the need to carry out scarification work once every 3 years.

The tillage systems influenced the grain wheat yield obtained per unit area, the values being different depending on the tested variants.

As a result, the conventional tillage system produced the maximum wheat yield (6169

kg/ha for the deep plowed scarified soil version) while the conservative method produced the lowest yield (5144 kg/ha for the experimental disk variant).

Under terms of TGW evolution, it fluctuated between 36.0 g of nonscarified soil under the conservative minimal work-disc approach to 38.4g on scarified soil when direct sowing was used. The scarified soil variant recorded values of 36.8 g for the wheat crop's one thousand grain weight, while the nonscarified soil variant recorded values of 37.4 g. The difference between the two tillage soil tillage variants was 0.6 g. Regarding the crude protein content in wheat grains, similar values were recorded in both tillage systems, 12.6% for the conventional system and 12.2% for the minimal tillage system.

The percentage values of starch content in the wheat grain ranged between 67.8% disk -70% direct sowing, both on nonscarified soil and between 68.5 and 69.1% on scarified soil.

The wet gluten showed positive values, almost all having values above the technical minimum of 22%. The exception is the direct sowing variant on nonscarified soil in which the gluten was at 21.6% very close to the normal limit. At the same time, all the works carried out on scarified soil were registered at the value of 25%, which means an increase in quality over the scarified variant.

The Zeleny index demonstrated, from the bakery quality point of view, values that fell within the good range (Zeleny index 38.9 ml). At the same time, it was found that in the conservative system, the direct sowing variant, the Zeleny index (34.5 ml) was in the satisfactory category.

The hectoliter mass MH kg/hl determined in both soil work systems (conventional and conservative), unscuffed and stubbled soil had average values of over 80 kg/hl, which qualifies the yields obtained in category I.

From an economic point of view, such values achieved in experience indicate obtaining higher prices per product unit.

Both positive and negative relationships were found among the analyzed indices. The correlations obtained between grain production with protein, gluten and TGW recorded insignificant positive relationships.

In the 2023-2024 agricultural year, we found that, in practice, the conservative method (disc, direct sowing) produced outcomes with comparable values to the conventional system (plowing) in terms of biomass and quality indices.

Considering these positive aspects obtained in the experimental field of the ARDS Pitești, it is recommended to promote the conservative soil cultivation system - disc harrowing and direct sowing - in wheat crop.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

REGARDING TRANSFER FACTOR OF SOIL POLLUTION WITH HEAVY METALS IN SOIL-PLANT SYSTEM

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Abstract

Heavy metals such as lead (Pb), cadmium (Cd), nickel (Ni), zinc (Zn), copper (Cu) present in large amounts in soil surface horizons are chemically stable compounds. They are complexed with various organic or inorganic ligands, which amplifies their toxicity. They, as we know, produce negative effects in different plant organs, and by transfer in the food chain produce different pathologies in animals and humans. The aim of the present work was to determine the transfer factor of lead (Pb) and cadmium (Cd) from soil to crop plants based on the concentrations determined for these elements. The transfer factor (TF) values were calculated for lead (Pb) and cadmium (Cd). Thus, the transfer factor for Cd was much higher than that for Pb, but the values do not exceed the reference value, namely 1.

Key words*: environmental impact, food chain, heavy metals, soil pollution, transfer factor.*

INTRODUCTION

Because of potential effects on food and environmental safety, research on the transfer of heavy metals from soil to plants has become increasingly important. Lead and cadmium are two examples of heavy metals that can accumulate in soils because of natural and anthropogenic processes, including mining, prolonged industrial emissions and excessive use of chemical fertilizers. One in the soil, these metals can enter the food chain via plant uptake; depending on physiological and ecological conditions, different species may show different levels of tolerance and accumulation capacity (Lăcătușu et al., 1998; Cordoș, 2003; Lăcătușu et al., 2007; Coman & Dăscălescu, 2008; Yadav, 2010; Roba et al., 2015; Hreniuc et al., 2020; Taro and Coman, 2021; Rusin et al., 2021; Filip & Coman, 2023; Filip & Coman, 2024a).

The species of the genus Prunus, namely the plum (*Prunus domestica* L.) is cultivated because its fruits are nutritionally rich, appreciated for their flavor, yields are high at low cost and there is an increased possibility of exploiting the fruit (from fresh consumption to jam, marmalade, distillate products by specific processes, etc.). Plums are also more resistant to shattering than the fruits of other species of fruit trees. The relationship between heavy metals and prunes can be described by the action of antioxidants. It is known that heavy metals when they enter the body can generate free radicals and reactive oxygen or nitrogen species that can damage DNA, proteins and lipids. The antioxidants in plums act as protective shields, neutralizing free radicals and preventing cell damage. Although, prunes also stand out as an ally against oxidative stress, and nature seems to offer ways to mitigate the negative effects of heavy metals (Mitre, 2001; Oprea & Ropan, 2010; Boškovic-Rakocevic et al., 2014; Stoica & Giurgiulescu, 2016; Giurgiulescu et al., 2017; Mitre, 2021; Rusu et al., 2023; Rusu et al., 2024).

Like other fruit tree species, the plum, through its root system, also absorbs heavy metals from the soil, which can influence fruit quality and pose potential health risks to consumers. Therefore, understanding the dynamics of heavy metals transfer from the soil to the different organs of these plants and their accumulation of is essential for our food safety.

Several studies in the literature have examined bioaccumulation trends in different crops, but there is still a relative lack of data on heavy

metal transfer rates to crop plants of the genus Prunus (Lațo et al., 2012; Mireclki et al., 2015; Mao et al., 2019; Prabasiwi et al., 2020; Mostafa et al., 2021; Lere et al., 2021; Oladele et al., 2023).

The present paper aims to quantify the transfer capacity of soil heavy metal pollutants in Prunus fruits from different private farms present in the Baia Mare Depression, more precisely in the area of glacis and contact piedmont. Detailed analyses were performed for 2 heavy metals, Pb and Cd.

MATERIALS AND METHODS

Characteristics of the studied area

The area of interest (polluted area) is a large one of approximately 588.8 km^2, in the Baia Mare Depression comparing with a cleaner area in the Maramureș Depression, namely the Sighetu Marmației area, which is of 111 km^2. From these perimeters, soil samples were taken from different sampling depths according to Order No. 184/1997 and plum samples according to Order No. 184/2001.

The Baia Mare Depression (Figure 1) represents the contact zone between the Someșan Plateau and the Eastern Carpathians, located on the southern flank of the Gutâi and Țibleș Neogene. From the altimetric point of view, the base level of the depression varies between 150 and 350 m, while from the morphologic point of view it has a bowl shape. The relief is varied, and this paper focuses on the Seini - Baia Mare - Baia Sprie piedmont glacis, towards the southern flank of the Igniș-Gutâi Mountains (Badea et al., 1992; Coman, 2006; Coman, 2010a; Ștefan et al., 2012; Marian et al., 2016).

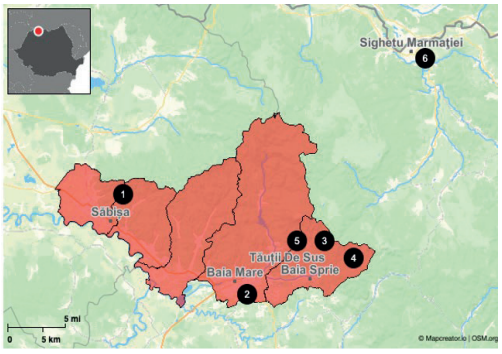


Figure 1. Sampling points in the Baia Mare Depression

As can be seen in Table 1, the minimum altitude is 252.9 m, and the maximum altitude is 356.4 m. All the farms are privately owned, well-kept properties.

Table 1. Sampling points

Point	Study area	Latitude	Longitude	Altitude (m)
1	Săbișa	47°44.1370'N	23°19.7050'E	270.9
2	Baia Mare	47°38.6340'N	23°35.3340'E	252.9
3	Baia Sprie I	47°39.8030'N	23°40.6890'E	343.9
4	Baia Sprie II	47°40.0090'N	23°40.5120'E	348.9
5	Tăuții de Sus	47°39.7710'N	23°37.1040'E	272.4
6	Sighetu Marmației	47°54.6710'N	23°56.8360'E	356.4

Working method

Soil sampling. Existing methodological regulations and standards, i.e. Order No 184/1997, are followed.

Soil samples were taken from the Baia Mare Depression (glacis and contact piedmont area) and Sighetu Marmației in the Maramureș Depression as a control sample. The soil profile was opened, and soil samples were taken from different depths, respectively 5, 30, 50 and 100 cm. After soil sampling, physic-chemical analyses were performed in an accredited laboratory, namely INCDO ICIA Cluj-Napoca according to ISO standards: SR ISO 11464:1994; SR ISO 11047:1999-PIS-03; SR EN ISO 1185:2009.

The values are compared with the legislation in force at national level, i.e. Order 756/1997, at European level, European Council Directive 86/278/EEC 1986, and at international level, (US EPA, 2002 after He at al., 2015b).

Fruit samples. Fruit sampling was carried out in August 2023 and August 2024, from the same locations where soil samples were taken. After sampling, the samples were transported following the transport rules and analyzed at the accredited laboratory INCDO ICIA Cluj-Napoca according to SR EN ISO 17294-2:2017.

The values are compared with the legislation in force at national level, namely Order No. 975/1998, at European level, namely Commission Regulation (EU) 1881/2006, Commission Regulation (EU) 629/2008, (EU) 2021/1317 completed by Commission Regulation (EU) 2023/915, and at international level, namely (Codex Alimentarius, 1995 and World Health Organization, 1996).

The transfer factor. In this paper, the transfer factor (TF) or in other papers titled as bioaccumulation index (BAF), biological accumulation factor (BCF) or bioconcentration factor (BCF_T) has been calculated according to the literature used at national and international level (Olănescu, 2007; Ivasiuc & Rusu, 2011; Big et al., 2012; Jolly et al., 2013; Nițu et al., 2019; Jakubus et al., 2019; Aladensami et al., 2019; Pehoiu et al., 2020; Noman et al., 2022; Tong et al., 2022; Pruteanu et al., 2022).

$$TF = \frac{Mp}{Ms},$$

where: TF - is the transfer factor (mg/kg);
Mp - represents the metal content in the plant (mg/kg);
Ms - represents the metal content in soil (mg/kg).

Regarding the TF values, on the one hand, if the TF value is >1 it means that the plant has accumulated elements, on the other hand, if the TF value is <1 it means that the plant resists to assimilate elements. Values close to 1 indicate that the plant is not affected by element. The calculation of the values assumes that there is a linear relationship between the concentration of an element in the plant and in the soil (Mihali et al., 2013; Emurotu & Onianwa, 2017; Thien et al., 2021; Olusegun et al., 2023).

RESULTS AND DISCUSSIONS

Soil sampling

The Pb content in total and mobile form at different sampling depths from representative points in the Baia Mare Depression (Săbișa, Baia Mare, Baia Sprie I, Baia Sprie II and Tăuții de Sus) and one point from Maramureș Depression (Sighetu Marmației) are represented in Table 2.

Sampling points – Pb

In *Săbișa*, the Pb content in total form at all depths is below the maximum permissible limits at international, European and national level. Thus, the soil at this point does not involve the intervention threshold. A small approach to the limit is at 30 cm. The Pb content in mobile form is below 0.10 mg/kg, but no comparison can be made due to lack of clear legislation.

Table 2. Pb content in soil

No. crt.	Sampling point	Depth (cm)	Pb content (mg/kg)	
			Total	Mobile
1.	Săbișa	5	35.0	0.10
		30	42.7	<0.02
		50	22.9	<0.02
		100	21.0	0.09
2.	Baia Mare	5	27.0	<0.02
		30	385.0	<0.02
		50	238.0	<0.02
		100	480.0	<0.02
3.	Baia Sprie I	5	211.0	0.68
		30	88.3	0.34
		50	35.0	0.28
		100	32.8	<0.10
4.	Baia Sprie II	5	261.0	0.60
		30	41.7	<0.10
		50	33.3	<0.10
		100	29.1	<0.10
5.	Tăuții de Sus	5	890.0	0.087
		30	141.0	1.20
		50	35.3	0.16
		100	37.7	0.17
6.	Sighetu Marmației	5	40.7	<0.02
		30	28.8	<0.02
		50	31.6	<0.02
		100	27.7	<0.02
7.	MAL RO[a]	-	50	-
8.	MAL EU[b]	-	50-300	-
9	MAL INT[c]	-	200	-

MAL - maximum allowed limit; RO – Romania; EU - Europe; INT – International; a - Order No. 756/1997; b - Council Directive 86/278/CEE 1986; c - US EPA, 2002 after He at al., 2015b.

In *Baia Mare*, the Pb content in total form at a depth of 5 cm is below the maximum permissible limits of the legislation in force, while at 30 cm the value is 7.7 times higher than the reference value. At 50 cm we observe a decrease to 4.8 times the reference value, while at 100 cm we have the highest value recorded, almost 10 times the legislation. Thus, we can state that the pollutant has migrated into the soil and now the limits are exceeded at depths between 30 and 100 cm. The content of Pb in mobile form shows the same value of less than 0.02 mg/kg, but it is not possible to make a comparison due to the lack of clear legislation.
In *Baia Sprie I*, the Pb content in total form, at 5 cm we have an exceedance of about 4.3 times

the maximum permissible limits, at 30 cm we have a decrease to 1.8-fold above the maximum permissible limit. As for the depth of 50 cm and 100 cm the limits are not exceeded. The Pb content in mobile form is between 0.1-0.68 mg/kg, but it is not possible to make a comparison due to the lack of clear legislation.

In *Baia Sprie II*, content of Pb in total form, at 5 cm depth we have an exceedance of about 5.3 times the maximum permissible limit, and at 30 cm, 50 cm and 100 cm the values are below the maximum permissible limits. The content of Pb in the mobile form is between 0.1-0.6 mg/kg, but it is not possible to make a comparison due to the lack of clear legislation.

In *Tăuții de Sus*, the Pb content in total form, at a depth of 5 cm, has the highest value recorded, over 17.8 times higher than the maximum permissible limits, while at 30 cm are recorded exceedances of about 2.8 times the legislation. The content of Pb in mobile form presents values from 0.087-1.20 mg/kg but it is not possible to make a comparison due to the lack of clear legislation.

In *Sighetu Marmației* (the control), the total Pb content is below the maximum permissible limits at all the depths where sampling was carried out. The maximum value was recorded at 5 cm, 40.7 mg/kg, while the national legislation stipulates 50 mg/kg. The mobile Pb content is below 0.2 mg/kg, but no comparison can be made due to lack of clear legislation.

Sampling point – Cd

Cd content in total and mobile form at different sampling depths from representative points in the Baia Mare Depression (Săbișa, Baia Mare, Baia Sprie I, Baia Sprie II and Tăuții de Sus) and one point from Maramureș Depression (Sighetu Marmației) are represented in Table 3.

In *Săbișa*, the Cd content in total form at all depths are below the maximum permitted limits at international, European and national level. The Cd content in mobile form is below 0.2 mg/kg, but no comparison can be made due to lack of clear legislation.

In *Baia Mare*, the content of Cd in total form at a depth of 5 cm is below the maximum permissible limits, while at 30 cm there is an exceedance of about 1.2 times the current legislation. At 50 cm the Cd concentration drops sharply to 0.83 mg/kg, indicating a significant reduction in Cd accumulation. At 100 cm it shows an exceedance of about 1.4 times the current legislation. The Cd content in the mobile form is below 0.3 mg/kg, but no comparison can be made due to the lack of clear legislation.

Table 3. Cd content in soil

No. crt.	Sampling point	Depth (cm)	Cd content (mg/kg)	
			Total	Mobile
1.	Săbișa	5	<0.33	<0.02
		30	<0.33	<0.02
		50	<0.33	<0.02
		100	<0.33	<0.02
2.	Baia Mare	5	1.73	<0.02
		30	3.47	0.03
		50	0.83	<0.02
		100	4.0	<0.02
3.	Baia Sprie I	5	2.50	<0.05
		30	1.60	<0.05
		50	1.20	<0.05
		100	1.17	<0.05
4.	Baia Sprie II	5	2.57	<0.05
		30	1.27	<0.05
		50	1.40	<0.05
		100	1.23	<0.05
5.	Tăuții de Sus	5	8.80	<0.05
		30	1.67	<0.05
		50	1.43	<0.05
		100	1.33	<0.05
6.	Sighetu Marmației	5	<0.33	<0.02
		30	<0.33	<0.02
		50	<0.33	<0.02
		100	<0.33	<0.02
7.	MAL RO[a]	-	3	-
8.	MAL EU[b]	-	1-3	-
9.	MAL INT[c]	-	0.48	-

MAL - maximum allowed limit; RO – Romania; EU – Europe; INT – International; a - Order No. 756/1997; b - Council Directive 86/278/CEE 1986; c- US EPA, 2002 after He at al., 2015b.

In *Baia Sprie II*, the total Cd content is below the national and European maximum permissible limits at all sampling depths but exceeds the international values. The Cd content in mobile form shows values below 0.05 mg/kg but it is not possible to make a comparison due to lack of clear legislation.

In *Tăuții de Sus*, the Cd content in total form at the level of the surface horizon, 5 cm, we have a 3-fold exceedance of the maximum permissible limits, while at 30 cm, 50 cm and 100 cm the national and European limits are not exceeded, only the international ones. The Cd content in mobile form shows values of less than 0.05 mg/kg, but it is not possible to make a comparison due to the lack of clear legislation.

In *Sighetu Marmației* (the control), the Cd content in total form is below the maximum permissible limits at all sampling depths. We currently have a common value of <0.33 mg/kg. The Cd content in mobile form shows the same value of 0.02 mg/kg, but no comparison can be made due to lack of clear legislation.

Fruit samples

The results on the total form of Pb in fruits, for the year 2023 and the year 2024, are presented in Table 4.

Table 4. Pb content in fruits

No. crt	Sampling point	Pb content in 2023 (mg/kg)	Pb content in 2024 (mg/kg)
1.	Săbișa	<0.04	<0.15
2.	Baia Mare	<0.04	<0.15
3.	Baia Sprie I	<0.04	<0.15
4.	Baia Sprie II	<0.04	<0.15
5.	Tăuții de Sus	<0.04	<0.15
6.	Sighetu Marmației	<0.04	<0.15
7.	MAL RO[a]	0.50	
8.	MAL EU[b]	0.10	
9.	MAL INT[c]	0.10-3	

MAL - maximum allowed limit; RO – Romania; EU – Europe; INT –International; a - Order no. 975/1998; b - Commission Regulation (EU) 2023/915; c - C. A., 1995, WHO, 1996.

In this study, for the year 2023, that the Pb concentration was of 0.04 mg/kg, well below the maximum permissible limits (0.1 mg/kg) according to European and international standards and 0.5 mg/kg according to national legislation.

For the year 2024, a significant increase in the Pb content can be observed, reaching 0.15 mg/kg. This value does not exceed the national maximum permissible limit, but in terms of international and European standards it is exceeded by 0.05 mg/kg.

The increase in Pb content in fruits is due to increased periods of drought in 2024 (315.69 mm), so less precipitation than in 2023 respectively, according to National Meteorological Agency (ANM, 2025).

The results on the total form of Cd in fruits for the year 2023 and the year 2024 are presented in Table 5.

This research found, for the year 2023, the Cd concentration was constant at 0.04 mg/kg, which is below the maximum permissible limit of 0.05 mg/kg imposed by national and European legislation. The values are compliant and do not raise food safety concerns.

Table 5. Cd content in fruits

No. crt	Sampling point	Cd content in 2023 (mg/kg)	Cd content in 2024 (mg/kg)
1.	Săbișa	<0.04	<0.08
2.	Baia Mare	<0.04	<0.08
3.	Baia Sprie I	<0.04	<0.08
4.	Baia Sprie II	<0.04	<0.08
5.	Tăuții de Sus	<0.04	<0.08
6.	Sighetu Marmației	<0.04	<0.08
7.	MAL RO[a]	0.05	
8.	MAL EU[b]	0.05	
9.	MAL INT[c]	1-1.3	

MAL - maximum allowed limit; RO - Romania; EU - Europe; INT - International,;a - Order no. 975/1998; b - Commission Regulation (EU) 2023/915; c - C. A., 1995, WHO, 1996.

For the year 2024, data indicate an increase of Cd to 0.08 mg/kg at all sampling points. These values exceed the national and European legislation (by 0.03 mg/kg), but in terms of international legislation, the fruit is safe for consumption.

The increase in Cd content in fruit is also due to increased periods of drought in 2024 (i.e. 315.69 mm) less precipitation than in 2023 according to ANM, 2025.

The transfer factor

The transfer factor for Pb and Cd was calculated with the literature formula for the total and mobile forms as specified in the methodology above. The Pb transfer factor, from soil to fruit, for the years 2023 and 2024, in total and mobile form, is shown in Table 6.

In *Săbișa*, the total transfer factor exhibited an increase in 2024 compared to 2023, ranging from a 3.74-fold increase at 100 cm to a 3.89-fold increase at 30 cm depth. A similar trend was observed in the mobile form, with a 3.75-fold increase at 5 cm, 30 cm, and 50 cm, and a 3.77-fold increase at 100 cm, indicating a relatively uniform rise across sampling depths. Exceedances of the transfer factor were recorded at 30 cm and 50 cm, while the mobile transfer factor exceeded threshold values at all depths in 2024.

In *Baia Mare*, a region historically affected by significant industrial pollution, the data from 2024 indicate substantial increases in

contaminant levels, reaching up to 6 times higher than those recorded in 2023.

Table 6. Transfer Factor -Pb

No. crt.	Sampling point	Depth (cm)	TF 2023	TF *mobile* 2023	TF 2024	TF *mobile* 2024
1.	Săbișa	5	0.0011	0.40	0.0042	1.50
		30	0.0009	2.00	0.0035	7.50
		50	0.0017	2.00	0.0065	7.50
		100	0.0019	0.44	0.0071	1.66
2.	Baia Mare	5	0.0014	2.00	0.0055	7.50
		30	0.0001	2.00	0.0003	7.50
		50	0.0001	2.00	0.0006	7.50
		100	0.0000	2.00	0.0003	7.50
3.	Baia Sprie I	5	0.0001	0.05	0.0007	0.22
		30	0.0004	0.11	0.0016	0.44
		50	0.0011	0.14	0.0042	0.53
		100	0.0012	0.40	0.0045	1.50
4.	Baia Sprie II	5	0.0001	0.06	0.0057	1.50
		30	0.0009	0.40	0.0035	1.50
		50	0.0012	0.40	0.0045	1.50
		100	0.0137	0.40	0.0051	1.50
5.	Tăuții de Sus	5	0.0000	15.00	0.0001	4.00
		30	0.0002	0.03	0.0010	0.12
		50	0.0011	0.25	0.0042	0.93
		100	0.0010	0.23	0.0039	0.88
6.	Sighetu Marmației	5	0.0009	2.00	0.0036	7.50
		30	0.0013	2.00	0.0052	7.50
		50	0.0012	2.00	0.0047	7.50
		100	0.0014	2.00	0.0054	7.50

Furthermore, the transfer factor in its mobile form exceeded acceptable thresholds in both years of the study. In 2024, a consistent 3.75-fold increase was observed across the entire soil profile when compared to the previous year, suggesting a uniform upward trend in the mobility and bioavailability of contaminants throughout the sampled depths.

In *Baia Sprie I*, transfer factor values in 2024 showed a substantial increase compared to the previous year, ranging from a 3.75-fold rise at 100 cm to a sevenfold increase at 5 cm depth. This gradient highlights a significant accumulation of contaminants in the upper soil layers. The mobile form of the transfer factor also demonstrated elevated values, increasing between 3.75 and 4.4 times at 5 cm, indicating enhanced contaminant mobility. Notably, exceedances of the mobile transfer factor were recorded only at the 100 cm depth in 2024.

In *Baia Sprie II*, at the surface horizon 5 cm, there is a 57-fold increase in 2024 compared to the previous year. On the one hand, at 30 cm and 50 cm we have a smaller increase of about 4 times in 2024. On the other hand, the transfer factor at the depth layer level at 100 cm has 0.37 times smaller decrease compared to the previous year. Regarding the mobile form, significant differences are found at 5 cm, a 25-fold increase, while at the rest of the depths we have a 3.75-fold increase compared to the previous year. In all samples analyzed from this point, the transfer factor in the mobile form in 2024 exceeds the reference value.

In *Tăuții de Sus*, the transfer factor values in 2024 exhibited a marked increase compared to the previous year, ranging from a 3.82-fold rise at 50 cm depth to a 5-fold increase at 30 cm. These results indicate a significant accumulation of contaminants, particularly in the upper soil layers. In contrast, the mobile form of the transfer factor showed a notable decrease at 5 cm, with values in 2024 being 3.75 times lower than those recorded in 2023. Overall, the increases in transfer factor were more pronounced in the surface horizons compared to deeper layers. Exceedances of the transfer factor for the mobile form were recorded exclusively at the 5 cm depth in both years of the study.

In *Sighetu Marmației* (the control), transfer factor values in 2024 increased approximately 4-fold compared to those recorded in 2023. Similarly, the mobile form of the transfer factor exhibited a consistent 3.75-fold increase across all sampling depths, indicating a uniform trend in contaminant mobility. Exceedances of the mobile transfer factor were observed in all analyzed samples during both years of the study. The transfer factor from soil to fruit for Cd for the years 2023 and 2024 in total and mobile form is shown in Table 7.

In *Săbișa* the transfer factor in both the form as well as the mobile form increased 2 times in 2024 compared to 2023. Exceeded values are recorded for mobile form in both study years.

In *Baia Mare*, an intensely polluted areal, the transfer factor in total form, at all sampling depths a 2-fold increase compared to the previous year, while the mobile form, records a uniform 3.75-fold increase. Overruns are recorded in both years of the study for the mobile form transfer factor.

In *Baia Sprie I*, there is a 2-fold increase compared to the previous year for both total and

mobile form. Overruns are only recorded for the mobile form for the year 2024.

Tabel 7. Transfer Factor -Cd

No. crt.	Sampling point	Depth (cm)	TF 2023	TF mobile 2023	TF 2024	TF mobile 2024
1.	Săbișa	5	0.1212	2.00	0.2424	4.00
		30	0.1212	2.00	0.2424	4.00
		50	0.1212	2.00	0.2424	4.00
		100	0.1212	2.00	0.2424	4.00
2.	Baia Mare	5	0.0231	2.00	0.0462	7.50
		30	0.0115	2.00	0.0230	7.50
		50	0.0481	2.00	0.0963	7.50
		100	0.0100	2.00	0.0200	7.50
3.	Baia Sprie I	5	0.0160	0.80	0.0320	1.60
		30	0.0250	0.80	0.0500	1.60
		50	0.0333	0.80	0.0666	1.60
		100	0.0341	0.80	0.0683	1.60
4.	Baia Sprie II	5	0.0155	0.80	0.0090	1.60
		30	0.0314	0.80	0.0479	1.60
		50	0.0285	0.80	0.0559	1.60
		100	0.0341	0.80	0.0683	1.60
5.	Tăuții de Sus	5	0.0045	0.80	0.0090	1.60
		30	0.0239	0.80	0.0479	1.60
		50	0.0279	0.80	0.0559	1.60
		100	0.0300	0.80	0.6015	1.60
6.	Sighetu Marmației	5	0.1212	2.00	0.2424	4.00
		30	0.1212	2.00	0.2424	4.00
		50	0.1212	2.00	0.2424	4.00
		100	0.1212	2.00	0.2424	4.00

In *Baia Sprie II*, on the one hand, a decrease in the transfer factor at 5 cm of 1.72 times is observed in the year 2024. On the other hand, at all sampling depths there is an increase of 1.53 times (30 cm) and 2 times (100 cm) in 2024 compared to 2023. For the mobile form, we have a 2-fold increase, with the baseline value being exceeded only in 2024.

In *Tăuții de Sus*, the transfer factor shows a 2-fold increase compared to the previous year, while at 100 cm a 20-fold increase is observed. Mobile transfer factor values are 2 times higher in 2024. Exceedances are recorded only in 2024 for the mobile transfer factor at all depths.

In *Sighetu Marmației* (the control) we have a 2-fold increase in transfer factor for both total and mobile form. There were exceedances for the mobile form in both years of study at all depths.

CONCLUSIONS

Heavy metals content in the soil. We observe exceedances of the maximum admissible limits for Pb in Baia Mare (30 cm, 50 cm, 100 cm), Baia Sprie I (5 and 30 cm), Baia Sprie II (5 cm) and Tăuții de Sus (5 cm and 30 cm). The highest value was recorded in Tăuții de Sus, and the limits exceeded in most samples was in the case of the Baia Mare sampling point, also known in the literature (Lăcătușu et al., 1996b; Ulmanu et al., 2006; Bora et al., 2020) as an intensely polluted area. For Cd in total form, we observe exceedances of the maximum permissible limits (in fewer cases than for Pb) at Baia Mare (30 cm and 100 cm) and Tăuții de Sus (5 cm) sampling points.

Heavy metal content in fruits. Concerning Pb content, the Romanian legislation is much more permissive (0.5 mg/kg) than the EU one (0.1 mg/kg), while for Cd the international legislation, namely Codex Alimentarius, is the most permissive (1-1.3 mg/kg), while both national and EU legislation (0.05 mg/kg) impose stricter maximum allowed limits. In 2023 there were no exceedances of the maximum allowable limits, but in 2024, the Pb content was of the international and European limit, but below the international threshold, and the Cd content was exceeded at the national and European level, but acceptable from the international point of view.

The transfer factor. The values in total form don't exceed the reference threshold (not greater than 1) which agrees with similar results reported by other authors (Mihali et al., 2013; Emurotu & Onianwa, 2017; Thien et al., 2021; Olusegun et al., 2023) and that a value greater than 1 was recorded in the case of the mobile fraction of Pb (Săbișa – 2023 - 30 cm and 50 cm, 2024 - all depth; Baia Mare – both years and both depth; Baia Sprie I – only at 100 cm in 2024; Baia Sprie II – 2024 – all depth; Tăuții de Sus – both years at 5 cm and Sighetu Marmației – both years at all depth) and Cd (Săbișa and Baia Mare and Sighetu Marmației – all depth and both years, Baia Sprie I, Baia Sprie II and Tăuții de Sus – only in 2024 at all depth).

Food safety remains an essential factor for both product quality and human health, thus maintaining its constant importance. At the same time, it is recommended to adopt a system of regular monitoring, since exceeding the maximum permissible limits can have negative effects on human health

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

THE IMPACT OF DCD AND DMPP ON NITRIFICATION IN ALKALINE AND SILTY LOAM TEXTURED SOIL

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Abstract

Nitrogen, which serves an important role in the vital activities of living organisms, is difficult to manage because it is highly mobile in soil. Nitrate, which is a form of mineral nitrogen, can cause economic and environmental effects through losses such as leaching and denitrification. Nitrification inhibitors slow down the biological process of nitrification in soil, preventing nitrate losses. In this study, the effects of DMPP and DCD on the nitrification process in soil with silty loam and alkaline textures were examined in an incubation conditions. The findings showed that the effects of DCD were still present on the 51st sampling day and both inhibitors were successfully inhibiting nitrification for more than 21 days. Soil mineral nitrogen content increased by 9% and 7% in the DCD and DMPP compared to the Control, respectively. DCD was found to be more effective at inhibiting nitrification in alkaline and silty loam soils. The impact of nitrification inhibitors on plant productivity and their function in mineral nitrogen losses require more research.

Key words*: DCD, DMPP, nitrogen, nitrification inhibitors, alkaline soil.*

INTRODUCTION

Nitrogen (N), one of the macronutrient elements for plants and microbes, is transported to animals and humans via the food chain (Blume et al., 2016). Plants and microorganisms get most of their nitrogen from the soil, but managing it is challenging because nitrogen is such a dynamic and mobile element (Zaman et al., 2009). For instance, organic N forms can be mineralized into ammonium (NH_4^+) and nitrate (NO_3^-) can be formed because of nitrification, and appeared nitrate can be either taken up by plants, leached from the soil, or removed from the soil with gaseous nitrous oxide (N_2O) by the process called denitrification (Gök et al., 1999). Due to the highly mobile nitrogenous compounds in soil, significant losses occur relatively quickly (Zaman et al., 2009). Losses in applied nitrogen fertilizers might result in greater agricultural inputs, groundwater pollution, or environmental issues such as global warming (Blume et al., 2016). There are different strategies for reducing these losses. Nitrification inhibitors (NI) are one of the simple, practical, and successful techniques among the available approaches.

In well aerated soils, nitrifiers rapidly convert ammonium to nitrite and subsequently nitrate (Gök et al., 2004). Therefore, the predominant form of mineral nitrogen (N_{min}) in agricultural soils is composed of nitrate (Liu et al., 2013). Nitrate is susceptible to leaching and loss in gaseous form. Nitrification inhibitors (NI) delay bacterial oxidation of ammonium ions by inhibiting the activity of *Nitrosomonas* spp. bacteria in soil for a particular time as 4 to 10 weeks (Kim et al., 2012; Liu et al., 2013; Sahrawat, 2008). Thus, ammonium in the soil may be utilized by plants while also protecting them against nitrate losses, either directly or indirectly.

The use of NI increases crop yield as well as nitrogen usage efficiency by reducing nitrogen losses in the soil (Abalos et al., 2014; Linquist et al., 2013; Liu et al., 2013) while also contributing to the minimized environmental pollution (Cui et al., 2021). In addition, using NI provides farmers with more flexibility by reducing the number of fertilizer treatments and allowing for a wider range of application timings (Grant, 2005; Trenkel, 2010). Inhibiting nitrate formation indirectly reduces nitrogen loss through denitrification (Chaves et al., 2006; Yang et al., 2016). In contrast, the

form of NH_4^+ releases ammonia gas (NH_3), particularly under alkaline soil conditions, and serves as an indirect source of N_2O (Kim et al., 2012).

Although there are several effective NIs, such as 2-chloro-6-(trichloromethyl) pyridine (nitrapyrin) and 3-methylpyrazole (3-MP), the most commonly used are dicyandiamide (DCD) and 3,4-dimethylpyrazole phosphate (DMPP) (Lan et al., 2018; Liu et al., 2013; Zaman et al., 2009). Both inhibitors inhibit the enzyme responsible for the oxidation of NH_4^+ to NH_2OH, delaying the production of NO_3^- (Guardia et al., 2018). DCD's low volatility, economics, and water solubility have made it widespread (Zaman et al., 2009). On the other hand, DMPP provides benefits such being heterocyclic, which means it is highly effective even at very low doses (Chaves et al., 2006; Weiske et al., 2001), having less negative effects related to ecotoxicity, and being successful in lowering N_2O emission loss and NH_3 volatilization (Liu et al., 2013).

The effectiveness of NI's is influenced by various environmental and soil factors (Akiyama et al., 2010). Although several studies have shown that soil pH has a significant impact on NI activity (Abalos et al., 2014), there have been a few investigations in alkaline soils, and the findings are conflicting. For instance, studies are showing that DCD (Cui et al., 2022; Guardia et al., 2018) or DMPP (Gök & Pamiralan, 2017; Zhou et al., 2020) are more effective in suppressing nitrification in alkaline soils (pH≥7.5). Nitrification occurs between pH 5.5-10.0, with 8.5 being the optimal pH. Nitrification decreases significantly when soil pH<5, but increases rapidly when pH>6 (Sahrawat, 2008). This existing information indicates that nitrification is a very important biochemical process, especially for alkaline soils. Therefore, determining NI efficiency in alkaline soil conditions will increase N use efficiency by reducing N losses.

This study was carried out to examine the effects of DCD and DMPP inhibitors on ammonium and nitrate fluctuation in alkaline and silty loam soil under incubation conditions. Our hypotheses for this research are that in alkaline and silty loam-textured soil, (I) NI's will slow down NH_4^+ oxidation, causing the less NO_3^- formation in the soil and (II) DCD will be more effective than DMPP in inhibiting nitrification.

MATERIALS AND METHODS

The experiment's soil was collected from a 0-10 cm depth in an area that is representative of the Çukurova Region in Adana, Turkey. Soil was air-dried at room temperature and then sieved through a 2 mm diameter sieve. Basic soil analyses were performed to determine the typical characteristics of the soil to be used for the experiment, and the findings are presented in Table 1.

Table 1. The characteristics of the soil used in the study

Properties	Unit	Value
Texture		Silty loam
Sand	%	20.7
Silt		53.1
Clay		26.2
Organic matter		2.01
$CaCO_3$		31.0
pH (1:2.5 H_2O)		7.84
EC	dS m^{-1}	0.12
NH_4^+-N	mg kg^{-1}	6.4
NO_3^--N		10.4
P		8.72
K		468
Fe		4.57
Zn		0.37
Mn		15.9
Cu		1.75

A 300 g of soil were weighed into containers of 5 cm height and 10 cm in diameter. As an ammonium source, $(NH_4)_2SO_4$ (Sigma 1.01217) was used. Dicyandiamide source was DCD (%99 DCD; Sigma D76609), and DMPP source was ENTEC Solub 21 (%0.168 DMPP + %21 NH_4^+-N) treatment. For the N+DMPP treatment, since ENTEC Solub 21 contains 21% NH_4^+-N, the remaining amount of N was provided with $(NH_4)_2SO_4$ to achieve a total of 300 mg N kg^{-1}. Treatments were Control (300 mg N kg^{-1}), N+DCD (300 mg N kg^{-1} + 30 mg DCD kg^{-1}) and N+DMPP (300 mg N kg^{-1} + 3 mg DMPP kg^{-1}). Because DMPP is recommended to be at concentrations ten times lower than DCD (Weiske et al., 2001), similar doses were employed in this incubation trial. During the experiment, the soil's moisture content was increased to 70% of the water holding capacity, and the decreased moisture was given back with pure water at regular

intervals. The 12 pots prepared following the above-described procedure were placed in an incubator set at 30°C.

Determination of mineral nitrogen in soils

The soil's NH_4^+-N and NO_3^--N concentrations were determined on the 1st, 8th, 21st, 51st, and 81st days after the incubation experiment. To determine NH_4^+-N and NO_3^--N, 10 g of fresh soil were extracted with a 1% $AlK(SO_4)_2$ solution. The NH_4^+-N content was determined by spectrophotometric measurement of the green-colored complex formed by nitroprusside salicylate with ammonium at 675 nm (DEZWAS, 1983). The NO_3^--N content was also determined by spectrophotometric measurement of the yellowish color formed by nitrate with sodium salicylate at a wavelength of 430 nm (Fabig et al., 1978). The concentrations were determined by taking into consideration the moisture content of the soil. The results are expressed in grams per dry soil.

Statistical analysis

This research was carried out according to completely randomized experimental design. Two-way repeated measures analysis of variance (ANOVA) was used in the statistical analysis, which was conducted using the OriginPro 2021 program. In addition, the Tukey test was used to compare mean differences. The data was graphed using the "Paired Comparison Plot" add-on in OriginPro 2021.

RESULTS AND DISCUSSIONS

Changes in NH_4^+-N concentration

The concentration of NH_4^+-N throughout the experiment is presented in Figure 1. The NH_4^+-N values determined in the sampling carried out just after the applications were lower than the initial NH_4^+-N values. This may have been due to the fixation of ammonium by colloids. On the second sampling day, the 8th day, the NH_4^+-N values in the control decreased radically, while the others decreased at levels not statistically different from the initial values. The NH_4^+-N values at the 21st sampling day, the differences between DCD and DMPP became apparent, and DCD was more effective than DMPP. This effect continued on the day 51. In the last sampling day, all values had decreased considerably and the differences between the treatments disappeared.

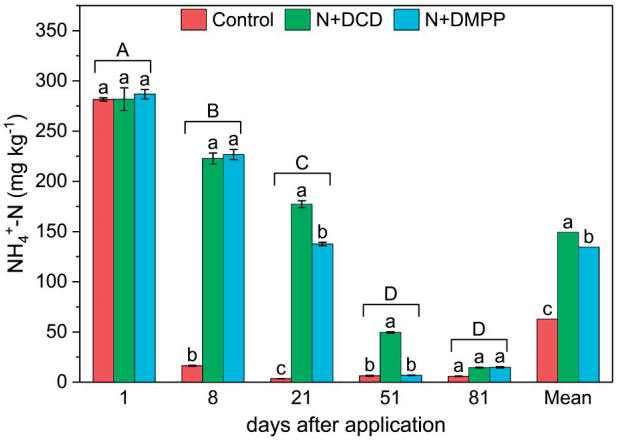


Figure 1. Effect of nitrification inhibitors on NH_4^+-N concentration in soil (According to the Tukey test, lowercase letters in the figure represent differences between treatments, whereas uppercase letters show differences between sampling days at a significance level of $p<0.05$)

According to these findings, NI's prevented nitrification more than 74% of the applied NH_4^+-N within the first 8 days and 46% on the 21st day. The Control treatment had 58% and 53% lower NH_4^+-N concentrations than the N+DCD and N+DMPP treatments, respectively ($p<0.05$). The obtained data are following the findings of Cui et al. (2021) that DCD and DMPP treatments had significantly ($p<0.05$) higher NH_4^+-N concentrations than the Control

treatment. When other studies on the subject were examined, it was determined that DCD and DMPP treatments were effective in suppressing nitrification in soil for 21 days (Guardia et al., 2018) and 35 days (Gök & Pamiralan, 2020). On the 21st day of sampling, where significant differences were found between all three treatments, N+DCD had 1.3 and 51 times greater NH_4^+-N concentrations than N+DMPP and Control treatments, respectively. Although there were similar concentrations between N+DCD and N+DMPP treatments, nitrification could not be suppressed in the Control treatment due to the absence of any NI and NH_4^+ was oxidized to NO_3^-. On this sampling day, 1%, 59% and 46% of the initially applied NH_4^+-N was retained for Control, N+DCD and N+DMPP treatments, respectively. Similarly, on the 51st day of the experiment, the highest NH_4^+-N value was obtained in the N+DCD treatment, with 6.7 and 6.3 times higher NH_4^+-N compared to N+DMPP and Control treatments, respectively. The results suggest that DCD is more effective than DMPP in suppressing nitrification in alkaline and silty loam soils. This might be attributed to the high dose of DCD used (10 times higher than DMPP). In soils without aeration problems, the dominant N form as a result of nitrification is NO_3^- (Gök et al., 2004), while with NI application, the dominant form became NH_4^+ for a certain period.

While N in the form of NH_4^+ in soils is in a form that is available to plants, it is protected against leaching by adsorption on clay minerals and organic matter due to its positive charges (Zaman et al., 2009), and N loss is indirectly prevented by denitrification (Abalos et al., 2014; Akiyama et al., 2010).

On the contrary, while there is concern that NH_4^+ preserved in the soil with NI applications may be lost as NH_3, especially in alkaline soil conditions (Kim et al., 2012), Yang et al. (2016) and Cui et al. (2021) reported in their studies that DCD and DMPP did not affect NH_3 loss.

Changes in the NO_3^--N concentration

The determined NO_3^--N concentrations were presented in Figure 2.

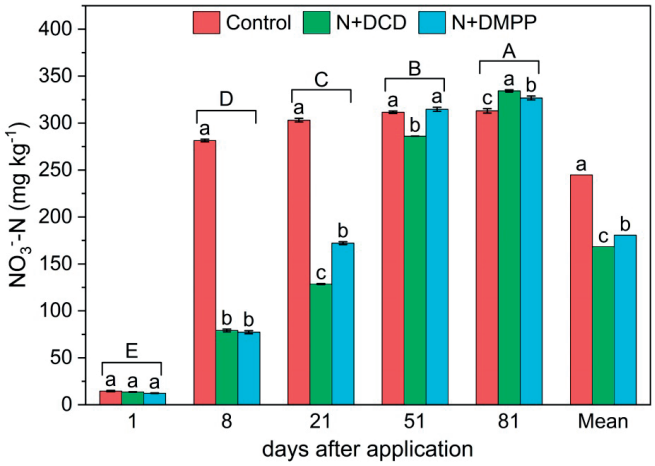


Figure 2. Effect of nitrification inhibitors on NO_3^--N concentration in soil (According to the Tukey test, lowercase letters in the figure represent differences between treatments, whereas uppercase letters show differences between sampling days at a significance level of $p<0.05$)

When the NO_3^--N averages of the soils for 5 sampling days were examined, the Control treatment had 34% and 24% higher NO_3^--N concentration than N+DCD and N+DMPP treatments, respectively, and it was found to be statistically significant among the treatments ($p<0.05$). According to the data obtained, the highest NO_3^--N was determined as 334 mg NO_3^--N kg^{-1} in the N+DCD treatment on the 81st day sampling and the lowest was determined as 12.2 mg NO_3^--N kg^{-1} in the N+DMPP treatment on the 1st day sampling.

Although there was no difference between the treatments on the 1st day of sampling, on the 8th day of sampling, the NO_3^--N content in all treatments increased by 19.3, 5.9 and 6.3 times for Control, N+DCD and N+DMPP treatments, respectively. Despite the application of NI in N+DCD and N+DMPP treatments, NO_3^--N contents increased by 65 mg kg^{-1} in the 8 days. The increase in the Control treatment was found to be 266 mg kg^{-1}, suggesting that NI's were successful in suppressing nitrification but were not entirely effective in preventing it. The results are compatible with research conducted by Gök and Pamiralan (2020), which found that both DCD and DMPP delay nitrification, and the impact is similar. On the 21st day, the Control treatment had 2.4 and 1.8 times greater NO_3^--N than the N+DCD and N+DMPP treatments, respectively. This difference was statistically significant ($p<0.05$). On the 51st day of soil sampling, NO_3^--N concentrations were similar for the N+DMPP and Control treatments, however, the N+DCD treatment had about 9% lower NO_3^--N, representing an inhibition effect continued. The findings are consistent with the results of Chaves et al. (2006), who found that DCD lost its effectiveness after 50 days, but contradict the findings that DMPP prevented nitrification for at least 95 days. The main reason for this may be due to the pH difference of the soils between the two studies. The soil pH used in Chaves et al. (2006) was 6.5, whereas the pH in this experiment was 7.84. Similar to the data in this study, Cui et al. (2021) reported that the effectiveness of NI's lasted for more than 110 days in acid soil (pH = 5.44), but this effectiveness decreased to 70 days in alkaline soil (pH = 7.66). According to the researchers, this was caused by the weakening of nitrification in acidic soils. Furthermore, Guardia et al. (2018) found that the nitrification inhibition efficiency of DMPP decreased when compared to DCD in low organic carbon and alkaline soil pH conditions. While the highest NO_3^--N concentrations in the 81st day sampling of the soils were determined in N+DCD and N+DMPP, these two treatments were determined to be statistically significantly higher ($p<0.05$) compared to the Control treatment. The low NO_3^--N concentration obtained in the Control application at the end of the experiment may be due to the fact that the dominant N form was NO_3^- as of the 8th day sampling and this NO_3^- was lost by denitrification (Abalos et al., 2014). Although denitrification is a reduction reaction that occurs in anaerobic conditions (Gök et al., 1998), N loss in the form of N_2O can also occur at water contents below 60% of the total pore volume (Blume et al., 2016). This could have occurred in N+DCD and N+DMPP, but since their NO_3^- concentration is smaller than in the Control treatment, there would likely be less denitrification loss.

Changes in the mineral nitrogen (N_{min}) concentration

The total mineral nitrogen as the sum of NH_4^+-N and NO_3^--N concentration values are given in Figure 3. According to the data obtained, the lowest and highest N_{min} concentrations were determined as 295 mg N_{min} kg^{-1} in the 1st day sampling and 349 mg N_{min} kg^{-1} in the 81st day sampling in the N+DCD treatments, respectively. There was no statistically significant difference between the N_{min} concentrations of the treatments until the 81st day of the study. On the 81st day, N_{min} concentrations increased significantly by 9% and 7% in N+DCD and N+DMPP treatments, respectively, compared to the Control treatment ($p<0.05$). This difference may have occurred due to possible mineralization of organic matter in favorable moisture content and temperature. The fact that both NI applications have higher N_{min} concentrations than the Control treatment suggests that N_{min} losses may have occurred in the Control treatment. This finding is consistent with the results of Linquist et al. (2013) that N losses in the pH range of 6-8 are reduced by the use of NI. In the Control treatment, the dominant N_{min} form being NO_3^- may have caused the difference between the treatments, resulting in N loss by denitrification. In a study conducted by Menéndez et al. (2012), dual application of fertilizer and DMPP reduced N_2O loss by 3 to 45% depending on incubation conditions compared to sole fertilizer application. In a meta-analysis conducted by Yang et al. (2016), it was stated that DCD and DMPP reduced N_2O at a similar rate (45% and 48%, respectively) and that this was due to the decrease in the NO_3^--N concentration of the

soil. During the experiment period, the N_{min} concentration of the soils increased in all three treatments. With the application of N+DCD, 67% of the added DCD contains N and as a result of the mineralization of 30 mg DCD kg^{-1}, an increase of 20 mg N kg^{-1} is expected in the soil. This increase may be the main reason why N+DCD has the highest N_{min} concentration. Furthermore, N mineralization in soils is expected to cause an increase in N_{min} concentration. In an incubation study conducted by Kaleem Abbasi et al. (2007), there was an increase of 24.4 mg kg^{-1} in the N_{min} concentration of the soil after 90 days in a soil with 0.85% organic matter.

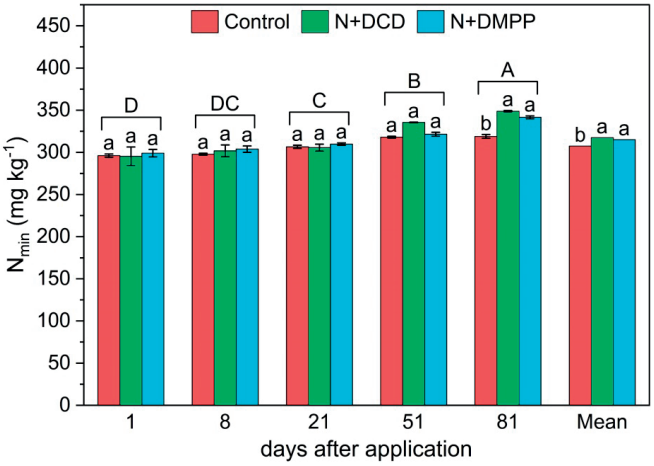


Figure 3. Effect of nitrification inhibitors on N_{min} concentration in soil (According to the Tukey test, lowercase letters in the figure represent differences between treatments, whereas uppercase letters show differences between sampling days at a significance level of $p<0.05$)

NH_4^+-N and NO_3^--N distribution among 5 sampling days

The distribution of NH_4^+-N and NO_3^--N in soils on 5 sampling days is given in Figure 4.

These data show that both NH_4^+-N and NO_3^--N are distributed homogeneously in NI treatments, but in the Control treatments, the distribution is predominantly in the direction of NO_3^--N.

This unbalanced distribution has the potential to cause NO_3^--N losses in soils.

On the contrary, the use of NI will ensure that NH_4^+ in the soil is converted to NO_3^- over time, thus keeping N stable in the soil and increasing N use efficiency (Akiyama et al., 2010).

CONCLUSIONS

Applications of NI's combined with fertilizer inhibited nitrification and maintained the soil's NH_4^+ content for over 21 days. This study shows that NI's are a significant tool to increase N utilization efficiency, particularly in soils with a risk of NO_3^- leaching. According to the findings, DCD is more advantageous than DMPP since it persists for longer than 51 days under alkaline soil conditions, is less expensive, and provides N to the soil through mineralization. Testing the real effectiveness of NI's in field conditions and examining their effects on plant parameters will be beneficial for farmers in the region.

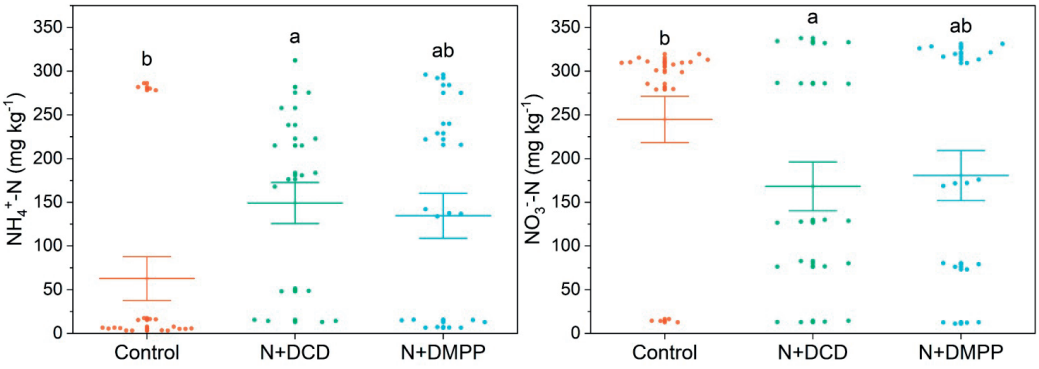


Figure 4. The 81-day distribution of the soil's NH_4^+-N and NO_3^--N concentrations
(Letters in the figure indicate differences at $p<0.05$ significance level according to Tukey test)

ACKNOWLEDGEMENTS

We are grateful to Prof. Dr. Ali COŞKAN for his thorough review of the manuscript, his constructive suggestions and improving the language.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

THE EVOLUTION OF MICROBIAL FUNCTIONAL PROFILE INVOLVED IN DECOMPOSITION PROCESSES IN LONG-TERM FERTILIZED EXPERIMENTS

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Abstract

The decomposition of organic matter plays an important role in the release and cycling of nutrients in agricultural soils. The use of 30-day decomposing straw technique, instead of the soil substrate itself, was chosen to highlight the microbial community involved in the decomposition of soil organic matter. EcoPlate (Biolog) readings were used to record daily changes in functional guilds. Dynamics of the Biolog EcoPlate showed the fluctuations within the functional microbial community active in the decomposition process. Both functional guilds presented a differentiated pattern over time. This functional analysis allows a better understanding microbial ability to break down and utilize the available compounds, underline their adaptability and metabolic diversity. The sum of activities recorded was more than 350% increase in NPK-amended variants. AWCD revealed a daily change in the performance of decomposing microbiomes with more than 525% increase in the same variants. Polymers, CH and AM guilds presented increases of over 400%. The interaction of microorganisms with organic matter is visible in the specific dynamics of functional microbiomes.

Key words*: functional microbiome, long-term impact, microbial dynamics, input-based fluctuations.*

INTRODUCTION

It was shown that soil stores twice as much carbon than the atmosphere (Dasgupta et al., 2024), making it the largest carbon reservoir on Earth (Devi et al., 2024; Juhos et al., 2021). But huge amounts of carbon have been lost from agricultural soil due to the application of an intensive technology requires its return in sequestered forms (Singh & Gupta, 2018). The global annual carbon losses are estimated at between 50% and 70%. (Dasgupta et al., 2024).

The agricultural sector is experiencing significant pressure due to the continuous growth of the world's population (Kopittke et al., 2019), which has led to a constant increasing demand for food. This demographic expansion requires a corresponding increase in agricultural yield, thereby exacerbating existing challenges within the sector (Fróna & Harangi-Rákos, 2019). Consequently, it has become imperative for agricultural practices to evolve and adopt sustainable solutions to effectively address the rising food requirements. The urgency of this situation highlights the need for innovation and adaptability in agricultural strategies to ensure food security for the growing population (Wang et al., 2024).

In addition, chemical fertilizers are an important part of modern agriculture (Kumar et al., 2019) but, in high quantities, they have a negative impact on the soil microorganisms and affect soil health (Pahalvi et al., 2021). These microorganisms work toward breaking down organic matter such as straw and turn it into the nutrients that the plants need to grow. Each type of soil microorganism has its own role, such as breaking down organic matter and supporting plant uptake of nutrients (Hayat et al., 2010; Nabi et al., 2024; Mo et al., 2024).

Currently, agroecosystems show significant potential to increase their organic carbon amount, and the recovery of lost carbon is a key to maintaining ecosystem functions. In this process soil micro-organisms play an important role (Singh & Gupta, 2018). They can use various organic compounds, such as carbohydrates, proteins, lipids and lignin, either individually or synergistically through the production of coupled enzymes during the decomposition process (Mohammadi et al., 2011; Wallenstein et al., 2013; Sigoillot et al.,

2012; Nicolás et al., 2019). Micro-organisms facilitate biogeochemical cycles, converting organic matter into forms accessible to other organisms and contributing to carbon sequestration (Shilky et al., 2023). It was shown that soil organic carbon (SOC) has increased in most cases with the return of plant residues (Castellano et al., 2015; Guenet et al., 2021) such as straw, into the soil for a long time. This technique aims to improve soil health and maintain fertility by increasing organic matter levels through the decomposition of straw. The incorporation of straw into the soil allows to sequester organic carbon which is a necessary part of sustainable agriculture (Fan et al., 2024). Straw are plant residues and represent a valuable agricultural resource that plays an important role in improving soil quality (Jin et al., 2020). They are a rich source of carbon, (Liu et al., 2014) and contain large amounts of nitrogen, phosphorus, potassium and micronutrients, which are important for crop growth (Xin et al., 2024). Their use as soil amendment has a high efficiency in maintaining and increasing the carbon (C) stock in arable soils (Saharan et al., 2024; Ji et al., 2024).

Wheat straw is an abundant and globally valuable resource of biomass with a structure composed of a mixture of natural polymers (Wang et al., 2017). Mainly wheat stalks cell walls contain cellulose, which is characterized by a linear and crystalline structure, providing rigidity and strength. Apart from cellulose, these cell walls also include hemicellulose, a non-cellulosic but complex polymer consisting of ramified chains of various sugars as well as lignin, which is a branched amorphous polymeric compound that contributes to the rigidity and hardness of the wall cell structure, but without crystalline organization. These components give wheat straw valuable physical and chemical properties, making it a useful plant material for multiple applications (Shanmugam et al., 2024). Straw acts as a bulking agent that increasing soil porosity, improving aeration and thereby promoting the efficient decomposition of organic matter in the soil (Ren et al., 2023).

The aim of this study is to evaluate the kinetics of functional microbiomes involved in wheat straw decomposition. The main objective was to monitor microbial activity over 24 h time intervals in 5 days (96 h), based on the activity recorded in incubated Biolog EcoPlates. The secondary objectives were to assess the changes in microbial community diversity and its ability to metabolize the different substrates. There were formulated two additional research questions: i) which carbon sources have shown the greatest effectiveness in stimulating the growth of microorganisms in fertilized versus unfertilized soil? ii) what are the observed trends in the use of carbon sources in Biolog EcoPlate in relation to the type of fertilization applied?

MATERIALS AND METHODS

Experimental design

The study was carried out in Research and Development Station for Agriculture Livada (RDSA Livada) Satu Mare County, Romania.

An experiment in 11 variants with 4 replicates (Table 1) each one was organized on a Luvisol type soil. Brown Luvisol is a common soil type in temperate regions (Lavkulich & Arocena, 2011), having a well-stratified structure and a clay horizon. In this soil, acid reaction can affect fertility and the availability of necessary plant nutrients. To correct this acidity and improve the conditions for plants, amendments like calcium carbonate ($CaCO_3$) are applied, which increase the soil pH. In our study amendments were applied using two different doses, 2.5 tons per hectare and 5 tons per hectare. Each dose of amendment was designed to help neutralize acidity and improve soil structure and fertility.

Table 1. Experimental variants and inputs applied over a long time

Variant	Amendment (t/ha)	Fertilizer (kg/ha)
V1	0	N0P0K0
V2	2.5	N0P0K0
V3	2.5	N100P0K0
V4	2.5	N0P70K0
V5	2.5	N100P70K0
V6	2.5	N100P70K60
V7	5	N0P0K0
V8	5	N100P0K0
V9	5	N0P70K0
V10	5	N100P70K0
V11	5	N100P70K60

For a complete analysis of input effects, these two types of doses of amendments are tested on

soils with different fertilization regimes. First, unamended and non-fertilized soils are compared as controls. Second, soil options treated with different types of chemical fertilizers are analyzed: nitrogen (N), phosphorus (P), an NP complex combining the advantages of nitrogen and phosphorus for optimal nutrient balance; and an NPK complex (nitrogen, phosphorus and potassium). Thus, by testing these amendment and fertilization combinations, it is possible to determine how each treatment contributes to improving the properties of brown luvisol, helping to increase agricultural productivity.

Decomposition protocol 30-day interval

The study of straw decomposition in soil involved the use of polypropylene bags (Ng et al., 2024), like tea bags. These bags are made from a fine, permeable material that allows microorganisms and air to penetrate, which are vital for decomposition process, but retains the plant material inside to facilitate observation.

Each bag contained 1 gram of wheat straw, dried at room temperature and ground to aid decomposition in experimental field. A total of 44 bags were placed in the soil, one in each of the 44 experimental plots at ARDS Livada, Satu Mare County.

Thus, 11 different variants were tested to provide a better understanding of the factors affecting the decomposition of wheat straw in the soil (Gheorghiţă et al., 2024).

The plant material decomposition experiment was conducted over a 30-day period, at a depth of 5-10 cm below the soil surface, near the root zone of the plants. This depth and positioning near the roots allowed direct contact with microorganisms around the roots, facilitating the observation of the decomposition process under natural conditions, directly influenced by the plant rhizosphere. The rhizosphere is known for the multitude of microorganisms that actively contribute to the decomposition of organic matter and to the nutrient cycle in the agricultural soil.

To maintain soil fertility and prevent nutrient depletion, experimental plots are cultivated using a crop rotation method. This involves changing the type of crop grown on the same plot each year. Through crop rotation, the soil maintains its nutritional diversity and long-term health, as each plant has different requirements and contributes its own organic substances to the soil. Thus, the experiment was conducted on plots with consistently fertilized soil, allowing for more precise monitoring of the decomposition process and the interaction between plant residues and the specific rhizosphere microbiome.

The protocol used for Biolog EcoPlate underwent slight modifications in terms of the material used. Instead of soil, which was traditionally used according to the method described by Garland & Mills (1991) and Garland (1997), decomposing wheat straw was used. This material originates from experiments carried out at ARDS Livada, Satu Mare County, where the bags were inoculated into the soil and left to decompose for 30 days. In the preparation stage, to realize the serial dilution, the decomposed plant material was processed until a dilution of 10^{-4} was obtained (Stoian et al., 2022). In addition, each well of the Biolog EcoPlate was inoculated with 120 µl of this dilution, thus adapting the method to the new experimental conditions. After inoculation, the plates were incubated at controlled temperature for the growth of microorganisms from the decomposed plant biomass. During incubation of these plates, microbial activity was monitored by measuring absorption, indicating utilization of carbon sources (Garland & Mills, 1991; Garland, 1997).

The substrates in Biolog EcoPlates represent different organic compounds - carbohydrates (CH), polymers (P), carboxylic acids (CX), amino acids (AA), and amines/amides (AM) that microorganisms metabolize for energy and growth, based on the method proposed by Stoian et al. (2022) and described as DEMSA concept. In this approach all chemical similar substrates are considered a guild, the term being used to describe an association of microbial groups with similar activities.

Analysis of microbial functional activity - in Biolog EcoPlate

The metabolic activity of soil microbial community was evaluated collectively using the Biolog EcoPlate, which measures their ability to utilize a variety of carbon sources.

The differences between organic and inorganic inputs (fertilized and unfertilized soils, as well as the effect of the amounts of amendments used), were analyzed by comparing the

percentages of different substrates use (as functional guilds) in 11 variants. The microbial evolution was monitored over periods of 48, 72, and 96 hours.

Measurements were made every 24 hours, starting 48 hours after inoculation and continuing up to 96 hours. The measurements were carried out using a spectrophotometer. With this device, the intensity of light absorption at different wavelengths was analyzed, as it provides precise information about the microorganisms in the analyzed substrates. This tool allows monitoring of the dynamics of microorganisms and other relevant factors for soil quality assessment, and the results help to compare the influence of different types of inputs on soil microbiome growth.

Data analysis

All data were analyzed in RStudio (version 2024.12.0; R Core Team, 2024). The package "agricolae" (de Mendiburu, 2023) was used to explore the differences between treatments, based on ANOVA and Least Significant Difference (LSD) test. The Nonmetric Multidimensional Scaling (NMDS) Ordination metaMDS in "vegan" package, (Oksanen et al., 2022; Corcoz et al., 2022) was performed to assess the interaction between functional guilds and treatments, and to explore the stability of functional communities within and between treatments.

RESULTS AND DISCUSSIONS

Changing the Biolog EcoPlate relevance for decomposition purposes

The Biolog EcoPlates analysis provides important information about the ability of the functional diversity of soil microorganisms (Jezierska-Tys et al., 2020) to metabolize different substrates present in the soil. The technique can be applied in long-term fertilized soils to assess the ability of soil microbial communities to use a set of substrates as a complex of species with similar metabolic activities. This analysis is important to better understand how microorganisms in different soils break down and use available compounds, highlighting their adaptability and metabolic diversity.

Based on the values after 48 hours of incubation the control variant (V1), showed the lowest values for all the functional guilds in Biolog EcoPlate profile. In particular, the lowest value was recorded for carboxylic acids (215%), followed by the amino acid guild, which had an increase of only 6% (221%), compared to CX, contributing to the lowest total value of activities (SUM). After the incubation period AWCD (Average Well Color Development) reaches 283%, indicating a high level of microbial activity. This value was based on the color intensity changes in EcoPlates, which reflected the overall metabolic activity of the microbial community in response to the available substrates. The results showed that a natural, unfertilized and un-amended soil does not significantly improve its quality or fertility in time, highlighting the limitations in the metabolic potential of the microbiota where the inputs are absent (Table 2).

After 48 hours the amines microbiome had the highest value of all the biochemical categories analyzed, reaching 458% in V3. This variant was fertilized with N and moderately amended with 2.5 t/ha of calcium carbonate. The carbohydrate functional microbiome, showed a 453% increase in activity value in the V10 variant, based on both amendment and complex application of NP (the position occupied by the polymer guild is higher in V4, which showed a 14% percentage reduction compared to the values recorded by the previous association.

For the basal respiration (Wat), the lowest value of 119%, representing almost half of the lowest levels observed in the other studied microbiomes, was recorded in V11. This variant was fertilized with NPK and strongly amended. The deficiency of carbon sources, even in the presence of nutrient inputs, can generate significant differences in the metabolic activity of soil microorganisms.

For a hierarchical analysis of amendment and input influence on microbial functional guilds, the values observed were classified separately for each functional type of microbiome. The basal community (Wat) showed increases due to the application of the medium levels of amendment, while the sum of the entire activity and AWCD showed fluctuations and reached higher values in highly amended variants that lack of fertilizer application. Carboxylic acid guild presented the maximum values in highly amended variants.

Table 2. Evolution of the functional guilds at 48, 72 and 96 hours of incubation as shaped by long-term applied inputs

	V	Wat %	Sum %	AWCD %	P %	CH %	CX %	AA %	AM %
48 h	1	154.86±15.67^{b}	240.67±35.12^{b}	283.55±52.41^{d}	280.48±44.60^{b}	279.65±51.22^{c}	215.07±27.56^{b}	221.73±24.68^{c}	249.77±46.48^{b}
	2	130.91±12.40^{b}	294.65±15.30^{ab}	346.67±27.03^{bcd}	344.59±11.06^{ab}	322.37±25.01^{bc}	280.09±21.39^{ab}	261.11±16.51^{abc}	295.41±38.55^{ab}
	3	236.70±76.91^{ab}	321.91±28.99^{a}	352.78±27.54^{bcd}	355.50±31.77^{ab}	317.32±32.38^{bc}	319.22±40.21^{a}	320.55±31.96^{ab}	458.11±40.18^{a}
	4	322.36±68.85^{a}	305.36±24.77^{ab}	299.13±21.49^{cd}	439.21±44.60^{a}	330.07±36.56^{bc}	285.08±31.65^{ab}	240.04±10.52^{bc}	329.49±32.21^{ab}
	5	203.05±43.37^{ab}	327.87±16.29^{a}	392.10±45.41^{abcd}	409.50±45.70^{a}	355.45±4.515^{abc}	302.02±38.69^{ab}	291.73±9.816^{abc}	355.63±43.53^{ab}
	6	250.32±139.23^{ab}	331.41±33.29^{a}	355.33±34.32^{bcd}	430.62±25.12^{a}	324.16±36.31^{bc}	311.76±33.49^{a}	347.48±43.57^{a}	326.08±27.85^{ab}
	7	138.46±27.23^{b}	363.92±31.90^{a}	525.36±119.31^{a}	386.48±37.00^{a}	400.62±38.67^{ab}	371.99±66.16^{a}	321.88±38.30^{ab}	389.28±59.30^{ab}
	8	160.16±51.36^{b}	340.96±20.51^{a}	421.22±31.67^{abcd}	385.11±29.28^{a}	391.08±20.10^{ab}	311.50±18.82^{a}	281.19±19.76^{abc}	424.47±68.08^{a}
	9	141.81±31.89^{b}	339.44±39.00^{a}	426.62±58.96^{abc}	404.20±27.75^{a}	388.98±39.80^{ab}	317.67±36.42^{a}	282.48±55.16^{abc}	302.33±40.62^{ab}
	10	129.70±20.31^{b}	343.72±16.58^{a}	434.89±38.16^{abc}	324.98±38.55^{ab}	453.16±36.41^{a}	304.51±28.22^{ab}	330.76±16.52^{a}	310.32±57.62^{ab}
	11	119.59±18.24^{b}	357.20±6.842^{a}	476.15±24.68^{ab}	421.84±13.86^{a}	364.15±22.09^{abc}	356.43±3.402^{a}	310.65±15.04^{ab}	398.49±104.19^{a}
72 h	1	174.07±22.50^{a}	148.55±2.42^{a}	145.92±3.79^{a}	167.92±13.59^{ab}	145.38±4.27^{ab}	144.24±1.82^{a}	157.94±5.81^{abc}	134.46±6.19^{abc}
	2	170.33±21.37^{a}	131.43±1.08^{ab}	126.81±3.19^{a}	135.75±3.22^{bc}	135.97±2.47^{ab}	126.32±2.70^{ab}	137.38±2.22^{bc}	109.10±3.20^{c}
	3	216.70±53.80^{a}	147.56±6.28^{a}	143.13±18.64^{a}	176.78±17.27^{a}	148.58±9.29^{ab}	132.34±4.06^{ab}	164.75±9.23^{ab}	139.28±13.97^{abc}
	4	221.05±113.90^{a}	143.80±2.54^{a}	140.74±18.09^{a}	139.00±3.51^{bc}	148.04±6.04^{ab}	131.07±3.41^{ab}	165.86±5.88^{ab}	149.60±13.60^{a}
	5	214.56±63.15^{a}	141.77±5.44^{ab}	130.52±6.34^{a}	135.25±1.39^{c}	143.22±9.60^{ab}	130.56±3.68^{ab}	166.33±7.08^{ab}	129.20±7.76^{abc}
	6	110.69±14.65^{a}	122.25±4.82^{b}	131.22±8.11^{a}	137.96±6.13^{bc}	124.36±2.95^{b}	114.80±5.09^{b}	127.47±8.38^{c}	110.73±12.26^{bc}
	7	199.63±46.33^{a}	143.31±14.67^{a}	131.07±9.16^{a}	154.31±16.49^{abc}	140.46±11.72^{ab}	142.65±17.50^{a}	152.33±16.89^{abc}	116.96±9.23^{abc}
	8	213.99±95.76^{a}	151.17±15.46^{a}	144.58±9.95^{a}	167.52±15.99^{abc}	149.60±20.89^{ab}	140.31±13.40^{a}	174.50±23.90^{a}	148.78±29.93^{ab}
	9	128.09±4.76^{a}	148.86±3.60^{a}	151.86±4.41^{a}	157.61±12.07^{abc}	148.92±8.04^{ab}	144.11±2.75^{a}	160.38±13.12^{ab}	127.44±13.99^{abc}
	10	154.60±19.15^{a}	140.81±1.15^{ab}	138.28±3.31^{a}	162.18±13.93^{abc}	140.49±3.26^{ab}	133.73±2.13^{ab}	153.23±6.96^{abc}	126.42±9.64^{abc}
	11	187.97±50.31^{a}	146.29±1.72^{a}	139.69±6.01^{a}	150.68±2.42^{abc}	152.96±5.93^{a}	140.56±2.04^{a}	156.08±6.05^{abc}	114.49±5.53^{abc}
96 h	1	131.75±8.22^{ab}	123.13±1.08^{ab}	121.32±1.40^{abc}	127.56±2.29^{ab}	121.67±1.88^{ab}	125.12±1.11^{a}	124.75±3.46^{a}	108.63±2.87^{ab}
	2	100.47±6.81^{b}	113.67±1.85^{bc}	115.87±2.25^{bc}	121.53±1.99^{b}	109.12±3.66^{bc}	113.48±1.19^{bc}	118.um19±3.78^{ab}	111.01±2.61^{a}
	3	113.50±9.26^{ab}	116.50±1.11^{abc}	118.35±4.02^{bc}	125.63±2.99^{ab}	115.48±2.53^{abc}	114.18±0.59^{bc}	120.18±2.68^{ab}	109.22±9.53^{ab}
	4	86.30±2.93^{b}	118.09±1.40^{abc}	135.77±9.72^{a}	128.23±4.11^{ab}	119.53±3.21^{abc}	114.67±1.16^{bc}	115.42±3.03^{ab}	115.85±4.27^{a}
	5	101.83±7.20^{b}	119.41±0.38^{ab}	127.28±4.94^{ab}	126.08±1.45^{ab}	115.26±1.46^{abc}	118.95±0.78^{ab}	123.32±2.86^{ab}	120.13±2.14^{a}
	6	141.93±15.33^{ab}	119.15±1.90^{ab}	117.18±3.07^{bc}	126.92±1.55^{ab}	114.34±2.82^{abc}	119.94±1.13^{ab}	123.92±3.83^{a}	111.50±4.04^{a}
	7	169.49±43.44^{a}	118.31±2.34^{ab}	116.16±6.10^{bc}	128.98±7.42^{ab}	120.35±4.88^{ab}	112.97±0.93^{bc}	119.85±2.62^{ab}	118.12±8.72^{a}
	8	104.08±30.66^{b}	107.95±8.06^{c}	114.20±5.86^{bc}	131.75±11.49^{ab}	105.06±10.74^{c}	105.00±6.42^{c}	111.91±7.03^{b}	89.18±17.25^{b}
	9	169.28±24.44^{a}	113.93±6.92^{bc}	109.72±8.18^{c}	127.19±3.07^{ab}	107.19±9.23^{bc}	114.52±8.38^{bc}	120.73±4.46^{ab}	116.80±6.19^{a}
	10	137.60±14.30^{ab}	122.76±1.79^{ab}	121.50±1.83^{abc}	134.59±3.28^{ab}	120.55±1.28^{ab}	122.04±3.12^{ab}	123.07±4.19^{ab}	120.64±4.75^{a}
	11	126.35±11.95^{ab}	125.47±2.40^{a}	127.88±4.16^{ab}	136.87±4.27^{a}	126.64±3.42^{a}	120.27±2.71^{ab}	125.90±4.17^{a}	114.89±5.19^{a}

Note: Means±s.e. followed by different letters indicate significant differences at $p<0.05$ based on LSD post-hoc test. Legend: V1 - control, V2-V6 - Am1, V7-V11 - Am 2, V2/V7 - 0N, V3/V8 - N100, V4/V9 - P70, V5/V10 - N100P70, V6/V11 - N100P70K60; Am1 - 2.5 T/ calcium carbonate /ha, Am2 - 5 T/ calcium carbonate /ha. Wat – basal respiration; Sum – total metabolic activity; AWCD – average well color development; P – polymers; CH – carbohydrates; CX – carboxylic acids; AA – amino acids; AM – amines/amides.

After 72 hours of incubation, the dynamics in functional microbiomes change quickly, and the lowest values are mainly seen in three of the five guilds analyzed (Table 2). In V6, the carbohydrate association reached 124%, the carboxylic acid microbiome 114%, and the amino acid microbiome 127%, due to complex treatments.

Basal respiration also recorded the lowest value of all guilds at 110%. In this variant, the combined value exceeds the basal respiration value observed at 72 hours by 12 percent.

As for the highest values, they varied between the different guild variants studied, reaching a maximum in the case of basal respiration in variant 4 (V4), at 221%. This variant was medium-amended and fertilized with phosphorus. The polymer microbiome shows the highest metabolic activity in variant 3 (V3), which was fertilized with mineral nitrogen and amended to a medium level, like the treatment applied in the previous case. A special case is observed within the carboxylic acid microbial association, where the same maximum value of 144% was recorded in two of the 11 variants analyzed: in the control variant (V1), but also in variant 9, which was fertilized with phosphorus and intensively amended.

The 72-hour analysis shows fluctuations in the basal respiration values, with similar results for the two doses of amendments applied (2.5 t/ha and 5 t/ha), with no significant differences between them. The total activity of guilds fluctuated, reaching a minimum of 122% in V6 (NPK fertilized and moderately amended) and a maximum of 148% in native soil (V1) and in V9 (Am2+P70). Within the guilds analyzed, the highest percentage value was recorded in variant V3 (Am1+N100) with 176% in the polymer microbiome, followed by 174% in variant V8 (Am2+N100) in the amino acid. These findings highlight the predominant role of nitrogen to support an active microbiome, whether the amendment intensity varies from moderate to high.

When read after a 96-hour interval, the amine values in V5 and V10 are similar, representing 120% of the NP-fertilized variant in both cases (Table 1.). The difference between these variants is determined by the amount of amendment used, which goes from a moderate to an intense level. However, these variations don't generate significant differences between the observed values.

The highest value is found in the WAT group, associated with basal respiration. Unlike the other variants analyzed, the absence of carbon sources contributing to these higher values. In this context, the percentage recorded 169% in the V7 and V9 variants, which represents a notable difference of 49% compared to the amine microbiome.

Variant V8 shows the lowest values recorded for four of the five guilds analyzed. This variant, characterized by intensive amendment and supplementation with nitrogen fertilizer, shows values ranging from 89% for the amine's guild to 105% for carbohydrates and carboxylic acids. On the other hand, in three of the five guilds studied, the highest values were observed in V11, characterized by intensive amendment and fertilization with an NPK complex. The polymer microbiome recorded a maximum of 136%, followed by an easy decrease of 10 percent in the case of carbohydrates. The amino acid microbiome showed 11 percent lower values compared to polymers, thus showing a distinct distribution of values according to treatment.

Basal respiration measurements for 96 hours showed higher values for Am2 compared to Am1, although there were small fluctuations. Percentage values of SUM varied from a minimum in V8 of 107% (Am2+N100) to a maximum of 125% in V11 (Am2+NPK). Intensive amendment had mixed effects, both reducing and increasing values, while nitrogen applied alone produced minor decreases and NPK complex increased microbial metabolism. All the measured results in the guild, values lower than 100% were detected. In V8 (Am2+N100), 89% were associated with the amine microbiome, indicating a possible inhibitory effect of the treatment.

Non-metric multidimensional scaling (NMDS) analysis has shown that the functionality of microorganisms involved in soil decomposition varies with the type of fertilization applied whether organic or inorganic over a long period of time as well as with the natural soil condition and agricultural land use.

The microbial community at 48 h shows fluctuations between untreated samples (Figure 1), without requiring specific inputs for carbohydrate and carboxylic acid vectors. A

precise transition to activities associated with basal respiration (WAT) is observed, as well as the functioning of the amine guild (AM). The guilds, including CH (carbohydrates), CX (carboxylic acids), AM (amines) and WAT (basal respiration), guilds demonstrate significant activity even in the absence of organic amendment or mineral fertilizer interventions. These findings suggest that undisturbed soil has the capacity to support a significant diversity of the microbial community.

The dominant microbiomes are associated with unfertilized soil, but the addition of organic inputs influences the specific accumulation of the microbial community. The intensity of these effects varies from moderate to pronounced and is particularly evident in soils under long-term fertilization. The intensive amendment with 5 t/ha of organic material, carried out without the application of mineral fertilizers induces a distinct change in the microbial community structure. This is visible in the position parallel to the 2-axis near carboxylic acid (CX) and carbohydrate (CH) vectors, without interacting directly with them.

In contrast, the NPK fertilizer complex, combined with an intensive amendment of 5 t/ha of carbonate, shows a marked effect on the microbial community, interacting directly with carbohydrates (CH), carboxylic acids (CX), amino acids (AA) and total microbial activities (SUM). This intersection indicates a significant influence of these factors on microbial functional guilds, suggesting that the simultaneous application of mineral fertilizers and organic amendments not only supports, but also actively shapes the structure and functioning of the soil microbial community. This highlights the importance of environmental conditions on microbial activity (Conant et al., 2011), and the continuous adaptation of the metabolic pathways used to decompose different organic materials. The relation with the environmental conditions is visible in the efficiency of the decomposition processes and in the composition and diversity of the microbial communities involved in this process. Therefore, studying these changes in microbial communities is a step forward to understanding the mechanism behind decomposition process (Chen et al., 2024). The combination of these inputs creates a favorable environment for enhancing microbial diversity and activity by stimulating metabolic processes associated with these vectors.

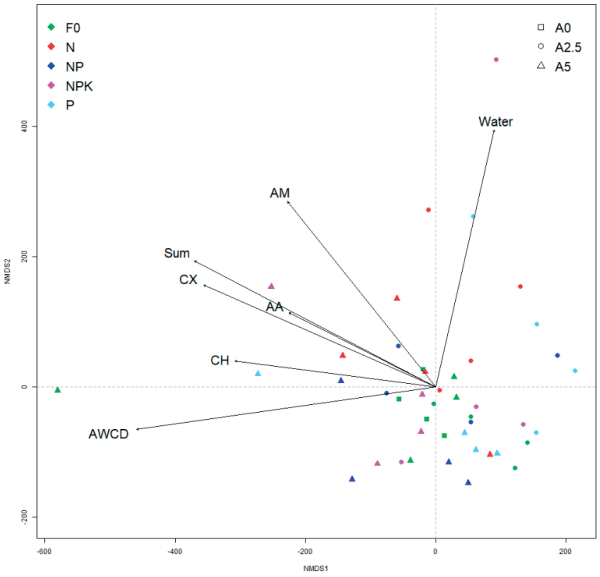


Figure 1. NMDS analysis of functional microbiomes after 48 h

In the 72-hour microbial diversity analysis (Figure 2), the distribution of the NP complex is characterized by a compact arrangement, indicating a uniform microbial diversity without

major differences. In addition to the use of NP fertilizers, the presence of organic amendments is also observed, whose intensity varies, like the previous scenario, from moderate to intensive. In this context, two amendment points intersect the vectors associated with basal respiration (WAT) and carbohydrates (CH). The analysis reveals important differences between intensively and moderately amended points, suggesting that intensive amendment contributes significantly to sustaining higher microbial diversity in soils subjected to long-term fertilization. This phenomenon highlights the positive impact of intensive amendment practices on structure and functionality of microbial community. Extensive research has shown that the populations, structures and metabolic rates of these microorganisms react differently to climate and technology changes. The diversity of microbial communities (bacterial and fungal) is also modified during the decomposition process (Das et al., 2007; McGuire & Treseder, 2010).

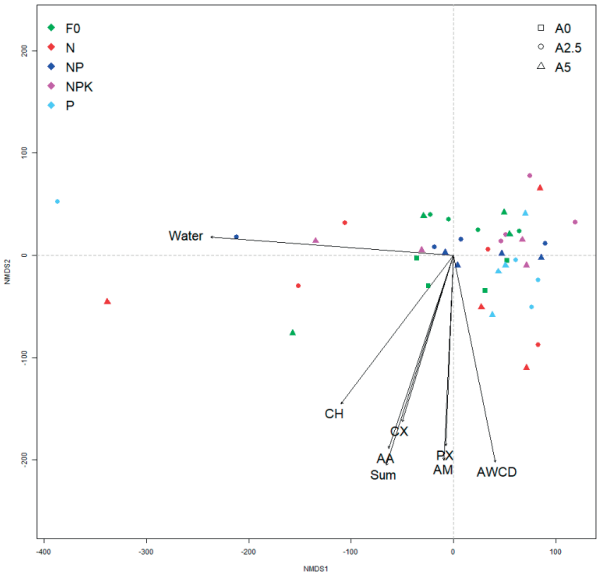


Figure 2. NMDS analysis of functional microbiomes after 72 h

At 72 hours, the lowest values are observed in variant 6, which is medium amended with 2.5 t/ha of calcium carbonate and fertilized with an NPK fertilizer complex. The values observed in this variant show a slight variation, ranging between 114, 124 and 127 percent, suggesting that the microbial diversity is relatively uniform, with no significant differences between the measured values.

The absence of mineral fertilizers results in a homogeneous microbiome with minimal functional differences. In this context, the absence of organic amendment correlates with the vectors of basal respiration (WAT), carbohydrates (CH), amines (AM), polymers (PX), and AWCD. These intersections suggest a less diversified microbial structure, characterized by restricted metabolic activity in the absence of amendment or mineral fertilizer inputs.

At the second reading, there is a clear connection and a significant functional similarity between the amine and polymer guilds (it was completely absent in the first NMDS). These guilds appear to be closely related to each other or react directly in the presence of unfertilized and unamended soil when the soil is in its natural state.

On the other hand, when phosphorus is added and intensive amendments are applied, a synergistic effect is created, which means that the diversity of micro-organisms increases considerably. This shows how soil interventions can modify and influence the relationships between microorganisms, sometimes amplifying their diversity. This suggests that

fertilization and amendment interventions can positively influence microbial diversity, especially in interaction with these functional guilds.

Nitrogen, used as a mineral fertilizer, interacts with functional guilds of basal respiration (WAT), carbohydrates (CH), carboxylic acids (CX), amines (AM), and AWCD. The N-associated microbial community dominates in this situation, being supported by moderate amendment, both in terms of basal respiration and carbohydrates, and in the assessment of total microbial diversity (AWCD). In contrast, intensive amendment with 5 t/ha of calcium carbonate exerts a significant influence on the microbial community, particularly affecting the guilds associated with carboxylic acids and amines, suggesting a more pronounced functional diversification in the presence of this intense amendment.

Analysis of the three carbon sources evaluated - carbohydrates, carboxylic acids and amino acids - shows that they show roughly similar values. However, the highest percentage was recorded in variant 8, in the amino acids microbiome, with 174%. This result is associated with the application of a high level of amendment, consisting of 5 t/ha of calcium carbonate, together with mineral nitrogen fertilization.

A positive relation can be observed between the intense level of amendment and fertilization and the increase in the values recorded in variant 8, particularly in the amino acid guild. In fact, variant 6, with a lower level of amendment and fertilization, showed lower values and a relatively constant microbial diversity over time. This contrast underlines the influence of fertilization and amendment regime on the measured values and thus on microbial diversity.

The microbial diversity assessed at a 96-hour time interval (Figure 3) shows a relatively uniform distribution of the focus points studied under long-term fertilization, indicating stability and equilibrium of the microbial communities under most of the conditions analyzed. However, there is a significant exception in the use of nitrogen fertilizer in combination with amendment at an intensive level, where notable differences in function are observed. These differences are evident both in comparison to the phosphorus-treated samples and to the native sample, which received no mineral or organic input, highlighting the distinct effects of fertilization on soil microbiota diversity and function.

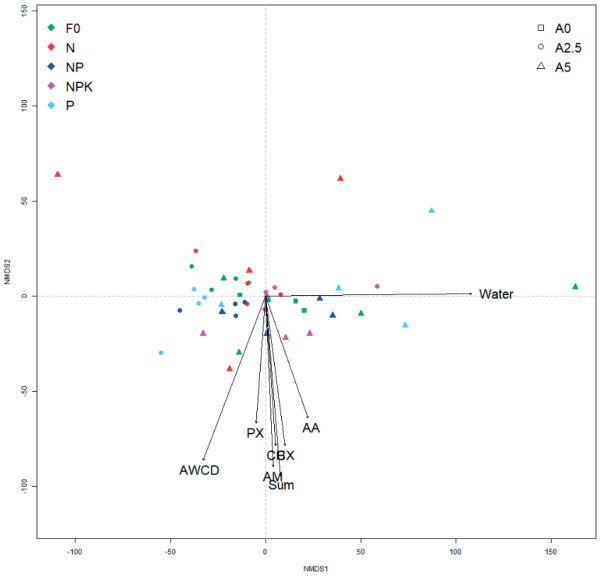


Figure 3. NMDS analysis of functional microbiomes after 96 h

Returning wheat straw back to agriculture can really help boost the soil micro-ecosystem and make this substrate more fertile (Li et al., 2024; Altieri, 2005). This method helps maintain the

right moisture and temperature in the soil, which is great for the organisms that live there. As straw breaks down, it provides important nutrients for the soil (Jin et al., 2020), and by adding straw back into the ground, farmers can improve natural fertility of their fields and help keep the soil ecosystem healthy (ZHANG et al., 2024).

The unfertilized variant, without the presence of amendments or with the addition of organic inputs such as calcium carbonate, shows a relatively uniform distribution of the points of interest on the vectors analyzed. In this context, the microbial community associated with undisturbed soil overlaps with the various functional measures, including basal respiration, amino acids, carbohydrates, amines and AWCD (total microbial community activity). Instead, intensive amendment in the absence of mineral fertilizer addition intersects with the same functional measures, such as WAT, AM, CH and AWCD, indicating a similar influence on functional microbial diversity. It is important to mention that amendment at a medium level is not detected in the studied guilds, being outside of them, and that it brings just minor changes in the functional microbiome of the experimental soil, emphasizing a low influence on microbial activity and structure in this condition.

CONCLUSIONS

Measurements at regular intervals showed a progressive decrease in values, with significant differences between 48, 72 and 96h. The microbial community involved in the decomposition of wheat straw acted rapidly, reaching a peak at 48h (458% in V3 - amines), followed by a considerable reduction at 72h (174% in V8 - amino acids) and at 96h (136% in V11 - polymers).

This development suggests the continuous use of available resources, and their depletion over time, on a general trend of decreasing values over time.

The 48-hour analysis showed a major increase in microbial activity, in the presence of inputs, determining an increase in the following functional guilds: amines, carbohydrates and polymers, which recorded values of over 400%.

After 72 hours, a change in the microbial guild dynamics was observed for amines polymer and carbohydrate, along with carboxylic acids and amino acid, all of them showing lower values.

Basal respiration is at its maximum level after 96 hours, indicating that the soil microbiome is performing at its optimum level in its natural environment under long-term fertilization.

The microbial community responds rapidly to changes in the environment by utilizing available nutrients and energy resources. As these are consumed, the micro-organisms adjust their metabolic activity, which can lead to a gradual decrease in biological processes dependent on existing carbon sources.

Based on NMDS analysis, the absence of polymer guilds at 48 and 96 hours suggests that other metabolic guilds are dominant at these time intervals, suppressing polymer utilization.

The NMDS projection at 72 hours showed the polymer guild intersection with the amine guild, indicating a similar microbial activity between the two guilds or even an overlap of the microbial communities responsible for the degradation of these compounds.

ACKNOWLEDGEMENTS

This work is part of a PhD study in the theme: “Methods to Isolate Decomposing Microorganisms from Agricultural Soils”, performed by the first author A.G., coordinated by prof. dr. Roxana Vidican (R.V.). Special thanks to the Agricultural Development Research Station Livada for their valuable support throughout this research.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

AGROGENETIC EVOLUTION OF ARABLE CHERONEZOMS IN THE AREA BETWEEN THE PRUT AND NISTER RIVER: STAGES, TRENDS

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Abstract

*The agrogenetic evolution of arable chernozems in the area between the Prut and the Dniester is examined through the prism of the genetic-evolutionary chain "elementary factors↔regimes↔pedogenetic processes↔soil" taking into account the changes induced in its components by the inclusion of soils in the agricultural regime. In such an approach, agrogenetic pedogenesis is examined through the prism of intercalated interdependent and interdetermined interactions induced by the use of soils in the agricultural circuit and climate change. In this context, the agrogenetic evolution of arable chernozems is genetically determined and the driving force of its development are the functional changes occurring in the organic matter system of so*ils.

Key words*: agrogenetic evolution, organic matter system, bioenergetic resources, energy potential, morphogenetic profile, humiferous profile.*

INTRODUCTION

The inclusion of soils in the agricultural circuit inevitably leads to changes in landscape conditions, and consequently in the soils as their basic component. In this context, it is unanimously recognized that the intensification of the agricultural production process has involved an anthrogenic-transformed trend in the evolution of soil resources, the proportions and meaning of which are determined by the regional bioclimatic and landscape conditions materialized in the type of pedogenesis and the initial state of the soils.

The processes of agrogenic transformation of soils, their functions and ecosystem services came into the attention of research in the seventies and eighties of the last century, being examined, in most cases, through the prism of soil degradation processes and the development of processes induced by agricultural use, especially by their cultivation, without taking into account the evolution of the pedogenetic environment determined by the intercalated action of natural and technoanthropic factors manifested in the pedogenetic (hydric, aeration, thermal) and pedofunctional (aerohydric, hydrothermal, oxidation-reduction, biological) regimes. Unidirectional changes in the pedogenetic process and soil functioning led to a unidirectional reduction in the natural fertility of soils, which led to the promotion of agrotechnical and agrochemical measures to increase "economic fertility" based on the simplistic agronomic approach to soil fertility, which is reduced to the intensification of work and the increase in the amounts of mineral fertilizers administered. The intensification of anthropogenic inputs led to increased yields under conditions of unidirectional reduction in soil fertility - a phenomenon that does not fit into the basic principles of the theory of pedogenesis. The evaluation of the anthropogenic-natural chernozem pedogenesis process through the prism of the "neodocuceavis triad" factor→processes→soil implied the conclusion that the reproduction of chernozem as a soil type under conditions of tillage is impossible (Gerasimov, 1983). The factorial-processual analysis of the chernozem process in the Carpathian-Pontian-Danubian space has highlighted a large group of natural and anthropogenic pedogenetic processes that manifest themselves in the intensification or inhibition of chernozem typogenetic processes (Lebedeva et al., 2013). At the same time, these lead to changes in the mechanisms of integration and structural-functional organization of soil matter (Jigău, 2003). The pedogenetic effects induced by these processes

materialize in some parameters of the pedogenetic and pedofunctional properties and regimes, as well as in features that have no anthropogenic analogues within the chernozem type of soil formation (Butenko et al., 2025; Cheverdin et al., 2016). Thus, the processes induced by the anthropogenic factor play an important role in the unidirectional increase in chernozem pedogenesis in the upper Holocene materialized in a new distinct phase - anthropopedogenesis - within the anthropo-natural trend of evolution of soils, soil cover and biopedoclimatic framework.

In this context, in a series of more recent publications the evolution of arable chernozems is examined through the prism of the neo-docuceavis triad (factors → elementary processes → soil (functions)) taking into account the evolution of pedogenetic and pedofunctional regimes as a result of the modification of the pedogenetic environment induced by the intercalated unidirectional impact of anthropopedogenesis and climate change (Jigău et al., 2018; Jigău, 2019; Jigău et al., 2023): factors ↔ regimes ↔ elementary↔ processes↔soilanthrofactors↔anthroregimes ↔anthroelementary↔processes↔ anthrosols

In such an interpretation, the interdetermined and interdependent interactions between the natural and the anthropogenic components are taken into account, which determine the meaning and intensity of the integrated process on agrogenetic pedogenesis, within which the ratio between typogenetic and anthropogenetic processes is determined by the concrete landscape conditions.

MATERIALS AND METHODS

The conceptual-theoretical framework of the present research is provided by the biophysical concept of pedogenesis according to which pedogenesis is the integrative product of the interdetermined and interdependent interaction of the constitution and integration of the physical, chemical, physico-chemical and biological subsystems of the soil ecosystem within the genetic-evolutionary chain: factors/ anthropofactors → regimes/anthroporegimes → processes/anthropoprocesses → soil/ anthrosol. Through this prism of ideas, the evolution of the soil ecosystem involves two distinct phases: a) the constitution of the routine (physical) soil system; b) the constitution and evolution of the soil ecosystem (Jigău et al., 2023). The constitution of the routine (physical) soil system is determined by the abiotic processes of transformation and organization of abiotic components (solid, liquid, gaseous) with the formation of the pedolithic profile, the structural organization of which ensures favorable conditions for the development of the living phase.

The constitution of the soil ecosystem is determined by the biologization of the pedomatrix with the formation of the biopedomatrix and its modeling by living organisms in accordance with their own needs (Florea et al., 2014; Florea, 2016; Kleber et al., 2007).

Through this prism of ideas, pedogenesis is a biophysical category and for its evaluation it is recommended to apply the parameters of physical and biological properties as well as the chemical and physico-chemical parameters related to them. In this context, in our research, the [agrogenetic system] is used as an integrative index of physical parameters and the [bioenergetic system] is used as an integrative index of biological parameters.

The conceptual methodological framework of the present research is ensured by the principle of the unity of the chernozem pedogenetic process in the natural regime and in the anthropo-natural regime. In this context, the evolution of chernozems in the space between the Prut and the Dniester in the anthropo-natural regime is determined by the complex of chernozem typogenetic processes (formation and accumulation of humus; aggregation-structuring of the soil mass; migration of carbonates) of biologization of abiotic matter and agrogenetic (mineralization of organic matter, dehumusification, dehumification, destructuring) of debiologization of the biopedomatrix (Aksenova, 2022).

The intercalated development of these two groups of processes materializes in the interdependent and interdetermined interaction of the pedofunctional system [bioenergetic system] ↔ [aggregate system].

To evaluate the parameters of the bioenergetic system, the indices of the composition of the organic substance system and the humic system

determined by the V. I. Tiurin method were used, and to evaluate the aggregate system, the indices of the structural-aggregate composition determined by the Savinov method were used (Kaurichev, 1980).

RESULTS AND DISCUSSIONS

In the present research, the evolution of arable chernozems under agrogenesis conditions is examined through the concept of genetically determined modification of chernozem typogenetic processes as a result of transformations in the system factors → regimes → elementary processes → soil as a result of the inclusion of lands in the agricultural circuit.

Through the laws of pedo-ecological relations (factors ↔ soil), the inclusion of chernozems in the agricultural circuit with all the related effects (substitution of natural vegetation with agrophytocenoses, deterioration of the "steppe litter" layer and the humic detritus layer, intensification of the degree of aeration of the arable layer, etc.) led to changes in the volume, composition and dynamics of the cycle of organic and biophilic substances in soils (Bruun et al., 2015).

The specified processes to a small extent affect the basic morphometric indices: the thickness of the humus layer (Am+Bm), the thickness of the humus-accumulative horizon (Am), the thickness of the humification-active transition horizon (Bm) (Table 1).

From the Table 1 presented, we note that during about 115 years the specified parameters have not undergone significant changes and the attested differences fall within the margin of error and, probably, are subjective in nature.

At the same time, however, there is a significant change in the thickness of the active root layer, which is caused, in our opinion, by the predominant concentration of nutritional elements in the upper and middle segment of the arable horizon. This entails a series of consequences manifested both in the quantity, establishment and dynamics of crop yields and in the meaning and intensity of the processes that take place in the "soil-plant" ecological system.

Table 1. Evolution of morphometric indices of chernozems in the area between the Prut and Dniester rivers in the post-Docuceavist period (1898-2011) (Jigău, 2023)

The soil	The soil	Morphometric index values					
		V.V. Docuceaev, 1898		I. A. Crupincov, 1988		Jigău, 2011	
		M	Max	M	Max	M	Max
Typical moderately humus-rich and leached black soils	Thickness of the humus layer (Am+Bm), cm	91	137	96	125	93	133
	Thickness of the humus-accumulative horizon (Am), cm	43	51	42	47	42	49
	Transition horizon thickness (Bm), cm	41.0	96	40	78	42	91
	Thickness of active root layer, cm	58	72	48	71	31	60

The concentration of crop roots in the upper and middle segment of the arable horizon has led to a reduction in the share of the edaphic volume of chernozems (85-95 cm) utilized by crop plants. As a result, the arable horizon is prone to depletion and the concentration of the root system in it significantly increases the degree of vulnerability of agroecosystems to drought. In a pedogenetic and functional context, this implies a significant reduction in the role of the root mechanism in the aggregation-structuring of arable chernozems.

At the same time, this implies a unidirectional reduction in bioenergetic reserves in the subarable segment (AmBm+Bm) of the arable chernozem profile. In addition, the increased degree of dynamics of the aerohydric and hydrothermal regimes, characteristic of the upper segment of the soil profile, leads to an increase in the share of the mineralization process within the integral process of mineralization-humification of organic residues and a reduction in the amounts of newly formed humus (Table 2).

Table 2. Composition of the organic matter system of arable chernozems under various maintenance conditions (layer 0-30 cm)

Mode of maintenance	Total organic matter content, %	Composition of the organic matter system, % of the total organic matter content in the soil		
		Humus	Non-humified organic matter	Mobile humic substances extracted in 0.1n NaOH
Leached clayey loam chernozem				
Hayfield	7.94	73.8	15.5	10.5
Lightly overcultivated arable land	4.98	83.9	7.2	8.9
Moderately overcultivated arable land	4.06	88.1	6.1	5.8
Heavily overcultivated arable land	3.29	93.7	2.9	3.4
Typical moderately humus-rich clayey loam chernozem				
Forest strip (47 years)	5.84	78.8	12.8	8.4
Fallow land (16 years)	5.39	71.1	13.7	10.2
Lightly overcultivated arable land	3.68	92.8	1.4	5.3
Moderately overcultivated arable land	3.08	94.8	0.9	4.3
Heavily overcultivated arable land	2.36	96.2	0.7	3.1
Typical weak humus-rich clayey loam chernozem				
Hayfield	5.91	77.1	14.5	11.4
Lightly overcultivated arable land	3.86	85.8	6.6	7.6
Moderately overcultivated arable land	3.00	90.7	4.5	4.8
Heavily overcultivated arable land	2.71	92.4	4.2	3.4
Clayey carbonate chernozem				
Wasteland	4.86	83.8	9.1	7.1
Weakly overcultivated arable land	3.02	88.9	4.3	6.8
Moderately overcultivated arable land	2.58	93.6	2.3	4.1
Heavily overcultivated arable land	1.83	95.3	1.1	3.6

From the table presented we can see that the use of chernozems in the agricultural circuit has led to the establishment of the overcultivation process in them, which involves changing the meaning and intensity of the overcultivation process, which involves changing the meaning and intensity of the chernozem pedogenesis process in conditions of pronounced deficit of bioenergetic resources. The quantity of the latter is reduced by about 1.6 times in slightly overcultivated soils, by 1.6-1.9 times in moderately overcultivated ones and by 2.2-2.7 times in strongly overcultivated soils.

From the perspective of the concept of "energy potential of soil organic matter", changes in the composition of the organic matter system imply a unidirectional change in the ratio between the components responsible for the share of three different forms of energy contained in the organic matter system: difficult to access contained in stable-inactive humic substances); moderately accessible (potential-active) contained in labile humic substances and microbial biomass; easily accessible (active) - the energy of non-humified organic matter (Masutenko, 2003).

In the context of the above, we mention that each component of the organic substance system has a certain role in its functioning and stabilization.

From the perspective of the concept of "energy potential", the fraction of stable humic substances (which has the major share in the

composition of the humic substance system) ensures the stabilization of the "carcass" of the organic substance system, but also the development of the spatial framework for the localization and implementation of the functioning processes of the organic substance system.

Labile humic substances enriched with nitrogen and easily mineralizable are an important source of energy accumulated in stable humic substances as well as of nutritional elements for plants and microorganisms. Thus, they contribute to the protection of stable humic substances from the impact of destruction-mineralization processes. At the same time, they are receptive to external impact, are prone to restoration and easily manageable.

Table 3. Energy potential of the organic matter system of chernozems in the area between the Prut and Dniester rivers under various conditions of layer maintenance (0-30 cm)

Maintenance mode	Energy potential structure: forms of energy						
	Total, gJ/ha	Inert		Potentially Active		Active	
		gJ/ha	%	gJ/ha	%	gJ/ha	%
Leached clayey loam chernozem							
Hayfield	6410	4731	73.8	994	15.5	685	10.7
Lightly overcultivated arable land	4020	3373	83.9	462	7.2	186	8.9
Moderately overcultivated arable land	3278	2888	88.1	200	6.1	190	5.8
Heavily overcultivated arable land	2650	2483	93.7	77	2.9	90	3.4
Typical moderately humus-rich clayey loam chernozem							
Forest strip	4715	3715	78.8	604	12.8	396	8.4
Fallow (16 years)	4352	3094	71.1	596	13.7	662	15.2
Lightly overcultivated arable land	2971	2757	92.8	42	1.4	172	5.3
Moderately overcultivated arable land	2487	2358	94.8	22	0.9	107	4.3
Heavily overcultivated arable land	1905	1833	96.2	13	0.7	59	3.1
Typical weak humus-rich clayey loam chernozem							
Hayfield	4771	3678	77.1	692	14.5	401	11.4
Lightly overcultivated arable land	3116	2674	85.8	207	6.6	235	7.6
Moderately overcultivated arable land	2422	2197	90.7	109	4.5	116	4.8
Heavily overcultivated arable land	2188	2022	92.4	92	4.2	74	3.4
Clayey carbonate chernozem							
Fallow	3924	3288	83.8	370	9.1	266	7.1
Weakly overcultivated arable land	2438	2167	88.9	10.8	4.3	163	6.8
Moderately overcultivated arable land	2083	1950	93.5	48	2.3	85	4.1
Heavily overcultivated arable land	1477	1408	95.3	16	1.1	5.3	3.6

Non-humified humic substances within the system of organic substances have two basic functions: 1) protective - by continuously providing the necessary nutrition and energy for the soil biota, it minimizes the mineralization processes of stable humic substances; 2) humus-reproductive - by directly participating in the humification processes. From the data presented in Tables 2 and 3, we find that the content of these two groups of substances undergoes a significant reduction in arable chernozems and as a result, a unidirectional trend of dehumification has been established in the latter.

As a result, a unidirectional trend of reduction of humus reserves and its fulvatization is established in arable chernozems (Table 4).

Table 4. Composition of the humic system of typical moderately humiferous chernozems (state 0-30 cm) under various maintenance conditions

Mode of maintenance	Carbon content % of total C											
	Total	Humic acids				Fulvic acids					C non-hydrolyzed residue, %	Cah/ Caf
		AH 1	AH2	AH3	Suma	AF1	AF2	AF3	AF4	Suma		
Forest strip (47 years)	2.68	11.8	27.5	3.9	43.2	1.9	6.7	15.2	2.8	26.5	30,3	1.63
Fallow land (16 years)	2.39	10.7	26.5	8.0	45.2	1.9	5.5	119	5.0	24.3	30.5	1.86
Lightly overcultivated arable land	1.98	14.0	19.7	6.2	39.9	6.6	7.9	10.0	4.8	29.3	30.8	1.37
Moderately overcultivated arable land	1.70	14.7	17.8	4.6	37.1	7.6	8.6	10.9	4.4	31.4	31.4	1.18
Heavily overcultivated arable land	1.32	14.9	18.1	4.6	37.6	7.8	8.8	9.8	4.9	31.3	31.1	1.21

From the data presented in Table 4, we find that in uncultivated soils (forest strip) the total organic carbon content (2.68%) is 0.70% higher than in soils with a low degree of overcultivation and 0.98% higher than in soils with a moderate degree of overcultivation. In soils with a high degree of overcultivation, the total organic carbon content is 1.36% lower than in uncultivated soils.

At the same time, the carbon content of the unhydrolyzed residue practically does not change. This allows us to conclude that the changes in the composition of the humus of arable chernozems are determined by the peculiarities of the agrogenetic pedogenesis process under conditions induced by the intercalated action of physical degradation and climate change (Khokhlova et al., 2014; Khokhlova et al., 2015).

The specified dehumification processes are not limited to the arable layer and extend to the AmBm and Bm transition horizons. At the same time, however, the processes of reproduction of humus reserves are mainly limited to the arable layer. In the underlying horizons, as a result of the reduction in the amount of bioenergy resources, insignificant amounts of humus are produced, which to a small extent compensate for the amounts of humus subjected to mineralization.

At the same time, the quantities of humic substances eluted from the arable layer to the underlying ones are significantly reduced.

As a result, in the lower horizons, compared to uncultivated soils, an uncompensated balance of humus is established.

In this sense, depending on the humus regime, the humiferous profile of arable chernozems is divided into two layers: a) arable with a quasi-balanced regime and balance of humus corresponding to the biohydrothermal and bioaerohydric conditions induced by the intercalated impact of agrogenesis and climate change: b) subarable - with an uncompensated-regressive balance as a result of humus mineralization. This implies the conclusion that under agrogenesis conditions the progressive-accumulative profile of native chernozems develops in a regressive-accumulative sense, and the trend of these changes is unidirectional with specific features depending on the soil subtype (Table 5).

Table 5. Elements of evolution of the humus profile of the chernozems of the area between the Prut and the Dniester in arable regime (native/arable)

Parameters	Chernozems				
	clay-iluvial	leachates	typical moderately humiferous	typical weakly humiferous	carbonate
Humus content in the 0-50 cm layer, %	4.23/3.75	5.04/4.06	4.90/3.98	3.60/2.65	2.88/2.41
Humus content in the 50-100 cm layer, %	2.26/2.29	2.40/2.10	2.17/2.17	1.52/1.06	1.39/1.35
Humus reserves, t/ha 0-50 cm	233/215	277/227	270/233	198/148	173/135
50-100 cm	124/143	144/131	130/136	91/66	76/71
0-100 cm	357/358	421/358	400/359	289/214	249/206
Evolutionary trend of the humus profile: compacted soil→arable soil	Accumulative with weak features of weak eluvial-iluvial differentiation → attenuation of the degree of eluvial-iluvial differentiation	Cumulative with weak features of progressive cumulative→uniform cumulative	Progressive-accumulative→more attenuated degree of progressive accumulation	Uniform accumulative with beginnings of regressive-accumulative→slightly regressive-accumulative features	Uniform accumulative with less quantitative expression→uniform accumulative with more expressed regressive-accumulative features

Despite the specified functional changes, the morphogenetic profile of arable chernozems retains the basic features inherited from the pre-agricultural stage. The unidirectional direction of the functional changes implies the conclusion of subsequent genetically determined transformations, over time, of the morphogenetic profile (Gromovic, 2024).

Elements of this are attested, by, at this stage and are manifested in the diversification of the basic features of the arable horizon (Table 6).

Table 6. Types of arable horizons characteristic of arable chernozems in the area between the Prut and Dniester rivers

Horizon, %	Humus content, %	Humus balance	Intensity of bioaccumulative processes	Degree of structuring	Aggregate stability
Agrocernic	› 6	Positive	High	Excellent	Excellent
Agromolic	5-6	Positive/balanced	Increased	Very good	Very good
Agrohumic	4-5	Balanced	High	Good	Good
Agroumbric	3-4	Unbalanced	Moderate/weak	Moderate	Moderate
Agro Greek	2-3	Severely unbalanced	Weak	Low	Low
Agrocenusius	‹ 2	Extremely negative	Very low	Very low	Very low

Based on the above, we consider that agrogenic evolution is genetically inevitable and its mitigation in order to preserve basic eco/agroecosystem functions and services requires differentiated management of soil bioenergetic resources (Almanova et al., 2023).

CONCLUSIONS

Along with the significant reduction in the total organic matter content in the soil, a stable trend of reducing the content of non-humified organic matter and mobile humic substances is also attested, and as a result, a unidirectional trend of dehumification has been established in the soils.

In the composition of the humic system, the total content of fulvic acids increases, and the Cah:Caf ratio presents values (< 1.4) that are not characteristic of the chernozems in the region.

The stable values of the hydrolyzed residue content imply the conclusion that the changes in the composition of the humic system of

arable chernozems are determined by the peculiarities of pedogenesis under conditions of agrogenesis and climate change. The humiferous profile of arable chernozems is clearly divided into two layers and indicates the substitution of the progressive-accumulative humiferous profile with the regressive-accumulative one, and the trend of these changes is unidirectional with specific features depending on the soil subtype.

The relatively short period of use of chernozems in arable regime was sufficient for the establishment, in the upper segment of the pedogenetic profile, of an arable horizon with features significantly detached from the humus-accumulative one.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

MICROBIOLOGICAL DIVERSITY AND CONTROL OF SOILS AND WATERS FROM FOREST AND AGROECOSYSTEMS UNDER DIFFERENT HYDROTHERMAL SOIL REGIMES AND AS FACTORS FOR HIGH-QUALITY AND SAFE COMPOSTING

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Abstract

Microbiological indicators of soils and waters from forest and agroecosystems have been studied. The main share in the composition of the soil microfl of microorganisms – actinomycetes and mold fungi, and an increase in anaerobes in over moistened soils have been established. In general, the total quantity of microorganisms is higher in agrogenic soils, which is also due to the fact that they are arable and with a controlled humidity regime. In these soils, a clearly higher quantity of microorganisms is established in spring compared to summer. The total microflora depends significantly on humidity, and the mineralization activity moderately on soil temperature. Pathogenic microorganisms have been established in some of the soils and waters, and for soils their quantity depends on the presence of pathogens in the waters that moisten them, as well as on other factors. Part of the soils were used as a substrate for composting, and part of the waters were used to moisten the compostable mixtures, after microbiological control.

Key words*: soil microflora, aquatic microflora, microbiological control, forest and agroecosystems.*

INTRODUCTION

Microorganisms are sensitive biomarkers characterizing changes and ongoing processes in any environment, in soils, compost and waters, in forest and agroecosystems, as well as in the composting phases. Soil and compost microflora depend on a complex of factors - temperature, humidity and pH of the substrate, mechanical composition of the soil, composition of the compostable mixture and other factors. The activity of microorganisms also depends on their quantity, but not as an independent factor, as well as on the composition of the microbiota.

The quantity and activity of soil microorganisms depends on soil temperature and humidity, as well as on the sampling depth, and based on these indicators, there are statistical models for recognizing and predicting soil microbiological activity by indirect signs, and it is determined that the most informative for indirect assessment of the number of soil microflora is the set of two diagnostic signs: sampling depth and soil temperature (Konsulova et al., 2017a,b,c), although microbiological indicators are highly dynamic, which determines the conduct of analyses in seasonal and annual dynamics. The moisture content of the compostable mixture is a dominant factor that affects aerobic microbial activity, to a greater extent than temperature, and the dependence between the two factors shows that the improvement of composting caused by an increase in temperature can only be achieved by increasing the moisture content (Liang et al., 2003). Moisture content of the compostable mixture 50-60%, monitoring of temperature in the composting phases, appropriate C:N ratio, pH, nutrient content are important factors for obtaining quality compost (Schulze, 1962; Suler and Finstein, 1977; Bertoldi et al., 1985; McKinley et al., 1986; Jackson and Line, 1997; Tiquia et al., 1998; Liang et al., 2003; Yordanova, 2020). Overwetting leads to a decrease in the amount of oxygen in the compostable mass and a slowdown in the rate of decomposition of organic matter (Lin, 2006). A similar trend is also found in overwetted soils (Naskova et al., 2015). In addition to the moisture content of the soil and compost, the microflora in the irrigation water also affects the soil and compost microbiome, and microbiological

control of the irrigation water should be carried out by testing for the presence of pathogenic microflora that could pass into the soil, compost and vegetation (Koleva et al., 2024). Similar microbiological control of water flowing to the soil, without a specific irrigation regime, is also carried out in forest areas. The problem is that the pathogenic microflora in soils is not standardized, while for water and compost there are regulatory documents with specified norms regarding pathogenic microorganisms such as *Escherichia coli*, *Salmonella* sp., *Enterococcus* sp., *Pseudomonas aeroginosa* and others. In agrogenic soils under different hydrothermal regimes and the influence of anthropogenic and environmental factors (irrigation, fertilization, meteorological conditions), as well as in the composting process, in general, the percentage of bacteria (non-spore-forming bacteria and bacilli) is higher than that of mycelial forms (actinomycetes and molds), and after irrigation the proportion of anaerobic bacteria increases, but their quantity remains lower than that of aerobes, and the quantity of molds, which are moisture-loving, also increases, as the additives used and their rate, and irrigation techniques affect the structure of the soil and compost microbial community (Malcheva, 2021; Malcheva et al., 2018a; 2018b; 2019a; 2019b; 2020; Naskova et al., 2015; 2016; Plamenov et al., 2016; Yankova et al., 2016; Frene et al., 2022; Guo et al., 2022; Koleva et al., 2024; Grigorova-Pesheva et al., 2024a,b). A similar percentage distribution of the main groups of microorganisms is also found in forest soils - the strongest development of non-spore-forming bacteria, followed by bacilli, and the least represented are actinomycetes and micromycetes (molds) (Bogdanov et al., 2015; Malcheva, 2020a; Malcheva et al., 2024a; Grigorova-Pesheva et al., 2024c). In soils after flooding, some authors found a higher amount of anaerobes compared to aerobes (Naskova et al., 2015). Some authors point to *Bacillus megaterium* and *Bacillus cereus* as the main representatives of the bacillary microflora (Malcheva, 2020b; 2020c; Malcheva et al., 2019a) in agrogenic and forest soils, other authors found an increase in the development of bacteria from the genera *Asticacaulis*, *Aquicella* and *Acromobacter* and an altered development of mold fungi - *Aspergillus* and *Alternaria* were reduced, while *Stagonospora* and *Metarhizium* increased in irrigated soils for 5 years in agriculture (Frene et al., 2022).

The aim of the study was to establish microbial diversity and conduct microbiological control in soils and waters from forests and agroecosystems, under different hydrothermal regimes and in the creation of high-quality and safe compost.

MATERIALS AND METHODS

Waters and soils from 4 regions of the Sofia Municipality were studied: village of Zheleznitsa, village of Pasarel, Vrazhdebna district, Vitosha Nature Park.

22 sites were analyzed, in seasonal dynamics (spring and summer) by soil indicators - at two depths (0-10 cm and 10-20 cm) - 44 soil samples per season, with and without influence from analyzed water bodies.

13 water bodies were analyzed - 1 mineral well, 1 natural spring, 2 artificial wells, 1 irrigation basin, 7 local water sources (water from fountains), 1 surface water source (river), where soils were also studied, with and without influence from the water source (Table 1).

Table 1. Soil and water variants

Variants (soils)
S1-Zheleznitsa - mineral drilling
S2-Zheleznitsa - to mineral drilling
S3-Zheleznitsa - natural spring
S4-Zheleznitsa - to natural spring
S5-Passarel, roadside fountain
S6-Passarel, to roadside fountain
S7-Passarel, to "Colorful fountain"
S8-Vrazhdebna, soil watered from a pool
S9-Vrazhdebna, soil watered from Probe 1
S10-Vrazhdebna, soil watered from Probe 2
S11-Vrazhdebna, soil watered from a fountain
S12-Vrazhdebna, unwatered soil
S13-Vitosha tulip, fountain
S14-Vitosha tulip, to fountain
S15-Vitosha, fountain
S16-Vitosha, to fountain
S17-Vitosha, "Stone fountain"
S18-Vitosha, next to "Stone fountain"
S19-Vitosha, Pavlova fountain
S20-Vitosha, next to Pavlova fountain
S21-Golden Bridges, Vladayska River
S22-Golden Bridges, next to Vladayska River
Variants (waters)
W1-Zheleznitsa - mineral drilling

W2-Zheleznitsa - natural spring
W3-Passarel, roadside fountain
W4-Passarel, "Colorful fountain"
W5-Vrazhdebna, watering pool
W6-Vrazhdebna, Probe 1
W7-Vrazhdebna, Probe 2
W8-Vrazhdebna, water from an outdoor fountain
W9-Vitosha tulip, fountain
W10-Vitosha, fountain
W11-Vitosha, "Stone fountain"
W12-Vitosha, "Pavlova fountain"
W13-Golden Bridges, Vladayska River

To prepare the studied compost, a compostable mixture of starter (soil and last year's compost), zucchini, dry and green weeds, whole plants of lavender, oregano, basil and thyme was used. Composting was done indoors, in a composter.

Microbiological soil analyses were carried out using the limiting dilution method and inoculations on solid nutrient media: meatpeptone agar (non-spore-forming bacteria and bacilli), Čapek-Dox agar (mold fungi), Actinomycete isolation agar (actinomycetes and bacteria that absorb mineral nitrogen) (Mishustin & Emtsev, 1989; Gushterov et al., 1977). The total microflora and the mineralization coefficient were determined by computational methods after enumerating the individual groups of microorganisms (Mishustin & Runov, 1957; Malcheva & Naskova, 2018).

Pathogenic microflora (coliforms and *Escherichia coli*, *Enterococcus* sp., *Salmonella* sp., *Pseudomonas aeroginosa*, *Clostridium perfringens*, molds and yeasts, total number of mesophilic aerobic microorganisms) in water was determined by applying membrane filtration and placing the filter on specific nutrient media for each pathogenic species. Cultivation was in a thermostat under aerobic conditions, with the exception of *Clostridium perfringens* - cultivation in a jar (anaerobic environment). The nutrient media and confirmatory tests are specified in the relevant regulatory documents: BDS EN ISO 9308-1:2014/A1:2017, BDS EN ISO 7899-2:2003, BDS EN ISO 19250:2013, BDS EN 26461-2:2004, BDS EN ISO 16266:2008, BDS EN ISO 6222:2002, BDS EN ISO 6222:2002.

The temperature of the samples was determined in the field using a temperature probe, and the pH was determined potentiometrically using a pH meter. The moisture content of the soil and compost samples was determined using a moisture balance, model DBS.

The statistical processing of the results included determining the average value of three repetitions and the coefficient of variation for the soil and compost samples. The correlation analysis was performed using the Excel 2010 program.

RESULTS AND DISCUSSIONS

The biogenicity of the studied soils in seasonal dynamics is presented in Tables 2 and 3.

Table 2. Quantity and composition of soil microflora (x 10^3 CFU/g; spring)

Variants	Depth, cm	Total micro-flora	Non-spore-forming bacteria	Bacilli	Actinomycetes	Micromycetes	Bacteria Assimilating mineral nitrogen
S1	0-10	1540	980	400	20	140	3040
	10-20	1460	960	360	20	120	3000
S2	0-10	2320	1700	480	20	120	3060
	10-20	296	224	58	2	12	342
S3	0-10	4700	3000	1600	20	80	4510
	10-20	536	360	148	20	8	475
S4	0-10	4300	2960	1260	20	60	4320
	10-20	510	350	132	20	8	466
S5	0-10	1340	860	400	0	80	2500
	10-20	192	136	50	0	6	272
S6	0-10	1360	900	380	20	60	2400
	10-20	186	132	48	2	4	264
S7	0-10	1360	880	400	20	60	2220
	10-20	148	100	40	2	6	236
S8	0-10	6320	5360	660	140	160	4850
	10-20	738	642	66	14	16	475
S9	0-10	6000	5200	620	120	60	4000
	10-20	618	532	66	12	8	420
S10	0-10	3880	3320	460	60	40	3850
	10-20	446	376	58	6	6	395
S11	0-10	4340	3880	400	40	20	3210
	10-20	448	400	40	6	2	334
S12	0-10	5560	5000	520	20	20	3020
	10-20	560	500	56	2	2	320
S13	0-10	3460	2800	540	20	100	4280
	10-20	2880	2400	400	20	60	3940
S14	0-10	3400	2760	540	20	80	4250
	10-20	516	390	100	10	16	484
S15	0-10	3080	2720	360	0	0	4830
	10-20	2620	2380	240	0	0	4080
S16	0-10	3080	2680	360	20	20	4750
	10-20	2700	2400	260	20	20	4500
S17	0-10	2980	2760	220	0	0	3900
	10-20	2520	2380	140	0	0	3420
S18	0-10	3020	2740	240	20	20	3860
	10-20	322	290	28	2	2	390
S19	0-10	400	280	120	0	0	620
	10-20	280	220	60	0	0	400
S20	0-10	1320	1000	280	20	20	1320
	10-20	150	112	34	2	2	148
S21	0-10	400	280	100	0	20	600
	10-20	360	240	100	0	20	460
S22	0-10	1880	1500	300	40	40	1120
	10-20	246	200	38	4	4	112

Table 3. Quantity and composition of soil microflora (x 10^3 CFU/g; summer)

Variants	Depth, cm	Total micro-flora	Non-spore-forming bacteria	Bacilli	Actinomycetes flora is occupied by non-spore-forming bacteria, followed by bacilli. Trends for weaker development of mycelial groups test	Micromycetes	Bacteria assimi-lating mineral nitrogen
	0-10	4520	4160	280	40	40	4150
S1	10-20	3200	3000	160	20	20	4150
	0-10	2240	1680	460	20	80	3000
S2	10-20	286	220	56	2	8	334
	0-10	5280	3000	2180	20	80	4510
S3	10-20	3010	2000	1000	2	8	3510
	0-10	4060	2800	1200	20	40	3830
S4	10-20	476	340	128	2	6	446
	0-10	1580	1100	400	0	80	2520
S5	10-20	164	112	40	0	12	260
	0-10	1600	1140	380	20	60	2460
S6	10-20	158	110	38	2	8	256
	0-10	1480	1000	400	20	60	2700
S7	10-20	158	104	46	2	6	278
	0-10	5900	5100	560	120	120	4300
S8	10-20	604	518	60	12	14	452
	0-10	5760	5060	580	100	20	3760
S9	10-20	600	520	66	12	2	389
	0-10	3680	3200	400	40	40	3550
S10	10-20	404	340	56	4	4	365
	0-10	4220	3800	360	40	20	3210
S11	10-20	450	390	54	4	2	332
	0-10	5540	5000	500	20	20	3020
S12	10-20	576	512	60	2	2	325
	0-10	3720	3000	600	20	100	5850
S13	10-20	3120	2600	440	20	60	4850
	0-10	3760	3040	600	20	100	5850
S14	10-20	398	316	68	2	12	608
	0-10	3120	2760	360	0	0	5550
S15	10-20	2200	2000	200	0	0	3760
	0-10	3260	2800	400	20	40	5780
S16	10-20	346	294	46	2	4	601
	0-10	3040	2800	240	0	0	4000
S17	10-20	2780	2600	180	0	0	3680
	0-10	3280	2880	320	40	40	4100
S18	10-20	362	308	42	4	8	418
	0-10	420	300	120	0	0	820
S19	10-20	400	280	120	0	0	700
	0-10	940	540	320	40	40	860
S20	10-20	136	74	52	4	6	92
	0-10	440	300	120	0	20	800
S21	10-20	400	280	100	0	20	720
	0-10	1300	1080	160	20	40	1000
S22	10-20	148	120	20	2	6	110

The main share in the composition of the soil microflora is occupied by non-spore-forming bacteria, followed by bacilli. Similar trends for agrogenic and forest soils are also established in other studies (Malcheva, 2021; Malcheva et al., 2018a; 2019a; Naskova et al., 2015; 2016; Plamenov et al., 2016; Yankova et al., 2016; Frene et al., 2022; Guo et al., 2022; Koleva et al., 2024; Bogdanov et al., 2015; Malcheva, 2020a). Trends for weaker and absent development of mycelial groups of microorganisms – actinomycetes and mold fungi in overmoistened soils are established due to the anaerobic conditions created. The mycelial structure of these groups of microorganisms and the fact that they are aerobic limits their development in an anaerobic environment. In most sites, the remaining groups of microorganisms also decrease in overmoistened soils, but less so compared to actinomycetes and micromycetes. Molds are acid-resistant and moisture-loving, but their development is limited in systematically overmoistened soils. In general, the total amount of microorganisms is higher in agrogenic soils from the Vrazhdebna district, which is also due to the fact that they are arable and with a controlled humidity regime. In these

soils, a clearly higher total amount of microorganisms is established in spring compared to summer – compared to uncultivated soils. The rate of mineralization does not depend solely on the amount of microorganisms (Malcheva, 2021; Malcheva et al., 2018a; 2019a; Naskova et al., 2015; 2016; Plamenov et al., 2016; Yankova et al., 2016; Koleva et al., 2024; Bogdanov et al., 2015; Malcheva, 2020a) (Figures 1 and 2).

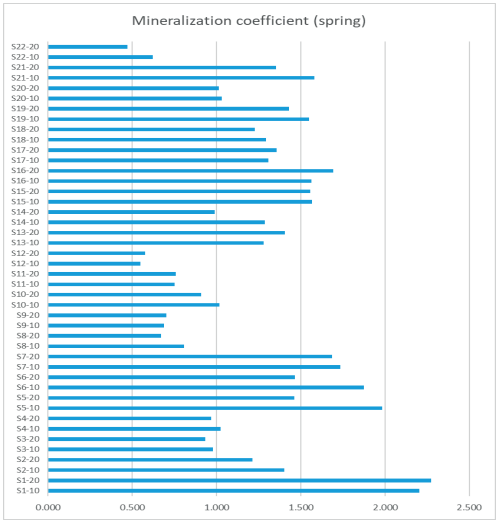


Figure 1. Mineralization coefficient values (spring)

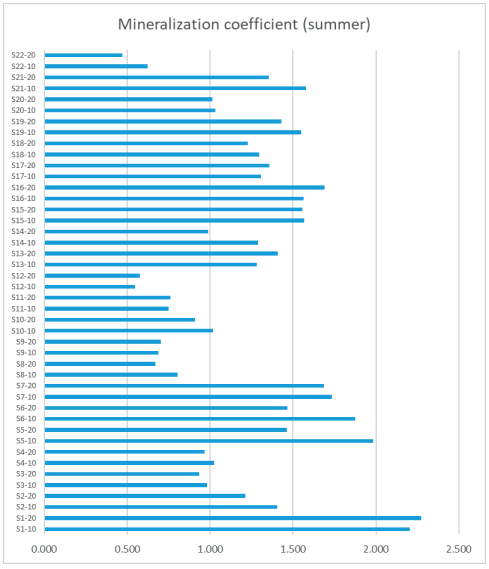


Figure 2. Mineralization coefficient values (summer)

The highest mineralization rate was found at S1 (Zheleznitsa, mineral drilling), and the lowest at S22 (Golden Bridges, next to the next to Vladayska River), which does not correlate with the highest and lowest total amount of microorganisms compared to all studied soils. A complex of factors has an influence: amount of microorganisms, temperature and humidity of the soils, mechanical composition of the soil, nutrients in the soils and other factors.

Soil humidity and temperature are interdependent factors affecting the soil microflora. When the soils are overmoistened, conditions are created for the development of anaerobic microorganisms and putrefactive processes. In soils after flooding, some authors found a higher amount of anaerobes compared to aerobes (Naskova et al., 2015). The soil humidity is higher at the sites of Vitosha, Pasarel and Zheleznitsa, where there is uncontrolled water inflow. At most of these sites, the amount of microorganisms generally decreases, especially for the mycelial groups of microorganisms, which are aerobic (actinomycetes, mold fungi). In spring, the temperatures in the arable soils from Vrazhdebna, as well as from the villages of Zheleznitsa and Pasarel, are slightly higher than in the soils from Vitosha. In the depth of the soil, the temperature decreases. The studied microorganisms are mainly mesophiles - in spring they develop at a temperature of the studied soils in the range of 9-15°C, and in summer - 28-32°C. In summer, the soil temperature increases, and the soil humidity decreases, which has the strongest impact on the development of the microflora in the arable soils. In the soils from Vitosha, Zheleznitsa and Pasarel, the soils have an acidic reaction, while the soils from Vrazhdebna have a neutral reaction. Microorganisms can also change the pH within limited limits. The more acidic environment creates conditions for more active development of mold fungi, but they are limited by the anaerobic conditions created in the overmoistened soils. The ratio of aerobes to anaerobes at different ranges of soil moisture is presented in Figures 3 and 4.

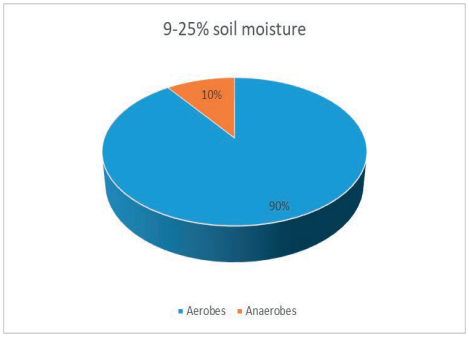


Figure 3. Aerobic: anaerobic ratio at soil moisture in the range of 9-25%

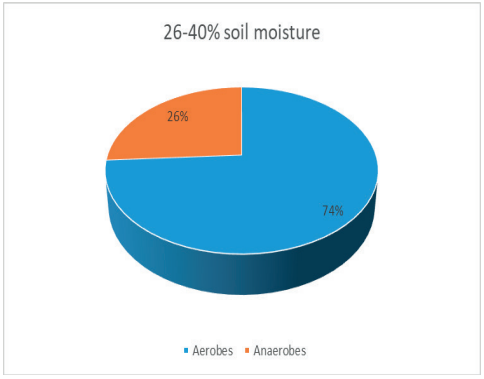


Figure 4. Aerobic: anaerobic ratio at soil moisture in the range of 26-40%

The correlations between the total microflora (TM), mineralization coefficient (MC), temperature (T), humidity (W) and pH of the soils are presented in Table 4.

Table 4. Correlation coefficients

Spring	TM	MC	T	W	pH
TM	1				
MC	-0.182	1			
T	-0.106	0.395	1		
W	0.569	0.109	-0.072	1	
pH	0.142	-0.465	-0.491	0.073	1
Summer	TM	MC	T	W	pH
TM	1				
MC	-0.276	1			
T	-0.015	0.394	1		
W	0.630	-0.218	-0.165	1	
pH	0.131	-0.624	-0.451	0.236	1

The total microflora depends significantly on the humidity and slightly on the pH of the soils, and the mineralization activity of microorganisms depends moderately on the temperature of the soils.

Pathogenic microorganisms have been identified in some of the soils, which mainly correlates with the presence of pathogens in the waters that moisten them, but in some sites other factors also have an influence - subsoil pollution, fecal pollution from animals, anthropogenic activity and other factors. In some of the waters, the presence of pathogenic microorganisms has been identified, mainly coliforms, *Escherichia coli*, *Enterococcus* sp., *Pseudomonas aeruginosa*. In the soils moistened by these waters, the same pathogens are found, without *Pseudomonas aeruginosa*. The microbiological control of different types of water is also carried out by the health inspections under a monitoring program. For the purposes of this report and in connection with a national composting project being developed, compost, with starter arable soil from sq. Hostile with the highest total microflora, as indicated, and watering in three variants to the thermophilic phase: with borehole water, pool water and tap water. No pathogenic microorganisms were found in the water from an external tap and from a borehole, while the presence of coliforms, *Escherichia coli* and *Enterococcus* sp. was found in the water from the pool. At the same time, the water from the pool contained the highest amount of total microflora of bacteria, actinomycetes and mold fungi, which are actively involved in the decomposition of organic matter in soil and compost. After composting, no pathogenic microorganisms were found in all three variants, and the total amount of microorganisms was higher in the variant watered with pool water, followed by the variant watered with borehole water and the total was lower in the variant watered with water from an external tap (Figure 5). The amount of non-spore-forming bacteria and bacilli was higher than that of actinomycetes and mold fungi in the composition of the total microflora in the studied compost. Similar participation of major groups of microorganisms in compost variants has been presented in other scientific studies (Malcheva et al., 2018b; 2019b; 2020; Koleva et al., 2024; Grigorova-Pesheva et al., 2024a; 2024b).

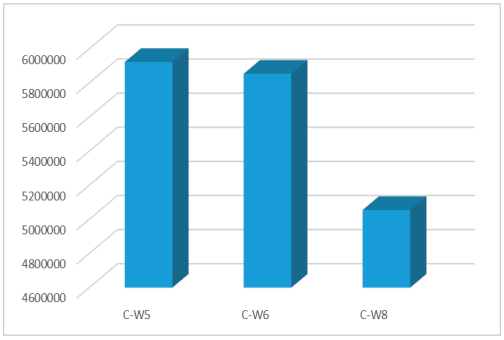


Figure 5. Total microflora in compost irrigated with water from a different source

On the one hand, the passage of the compost through a thermophilic phase disinfects it, but on the other hand, when the temperature increases, mesophilic microflora dies. The decomposition processes continue with thermophilic microorganisms. Temperature, humidity and pH have a major impact on the development of microorganisms in soils and compost. The amount and composition of the microflora in the starter, the type of waste used as a composition of the compostable mass and the irrigation water also have an impact on the production of high-quality and safe compost. The essential oil crops used (*Lavandula angustifolia* Mill., *Origanum vulgare* L., *Ocimum basilicum* L., *Thymus vulgaris* L.) have an antibacterial effect against *Escherichia coli*, *Enterococcus faecalis* and other pathogenic microorganisms (Man et al., 2019; Malcheva et al., 2024b; 2024c).

CONCLUSIONS

The total microflora is higher in agrogenic soils compared to forest soils, which is also due to the fact that they are arable and with a controlled humidity regime, with clearer trends for a higher amount of microorganisms in spring compared to summer. The rate of mineralization does not depend solely on the amount of microorganisms. The total microflora depends significantly on humidity and weakly on the pH of the soils, and the mineralization activity of microorganisms depends moderately on the temperature of the soils.

Bacteria predominate over the mycelial groups of microorganisms, with the amount of mold fungi increasing in soils with higher humidity, but not in overmoistened soils, in which the proportion of anaerobes increases.

In some of the waters, the presence of pathogenic microorganisms was detected, mainly coliforms, *Escherichia coli*, *Enterococcus* sp., *Pseudomonas aeruginosa*, which are also found in the soils moistened by these waters (with the exception of *Pseudomonas aeruginosa*). Watering the compost variant with water containing pathogenic microflora does not lead to the content of pathogenic microorganisms in the finished compost, they die during the thermophilic phase, but enriches the compost with bacteria, actinomycetes and mold fungi, which actively participate in the decomposition of organic matter. The essential oil crops used with antibacterial effect also have an effect on the destruction of pathogenic microflora.

ACKNOWLEDGEMENTS

This research work was carried out with the support in the framework of “Investigating interrelationships in different variations of organic waste composting, by creating dynamic compost mixtures with the addition of ameliorants and tracking microbiological and enzyme activity - variants for applying a quality organic-mineral compost-based improver in agricultural practice – КП-06-Н66/10” funded by Research Fund of Ministry of Education and Science in Bulgaria.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

STUDY ON LAND SUITABILITY IN BUCU AREA, IALOMITA COUNTY, FOR FORESTRY USE

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Abstract

The studied sector is deficient in terms of forest vegetation, with an obvious tendency to increase the average annual temperature, especially the summer ones. In order to reduce the effects caused by climate change, biodiversity conservation and implicitly the protection of agricultural land, it is intended to establish a forest on an area of 55.6 ha., with forest species suitable from a pedoclimatic point of view. As a result, a complex pedological study was carried out, in different plots, by performing a soil profile and several control surveys, in order to establish the suitability of the land for forestry facilities. The soil type identified is calcaric fluvisols, formed in Ialomita meadow, on account of alluvium, with flat configuration, clay-sandy texture and contrasting distribution. Based on these local pedoclimatic conditions, the formula for afforestation with xerophyte species was established, with fast growth and low requirements in terms of soil trophicity.

Key words*: shelterbelts, mixed forest, species composition, calcaric fluvisols, planting schemes.*

INTRODUCTION

Forest curtains are areas planted with different species of trees and shrubs, according to a well-designed scheme, with the aim of forming a barrier against winds that manifest themselves at the soil surface. They provide wind protection for homes, highways, agricultural land and represent a diversity of habitats in which different species of wildlife find shelter (Enescu, 2018; Giurgiu, 1995). This role of biodiversity is of great importance, contributing to a natural balance between pests and beneficial species, while enhancing the biological control of pests in agricultural crops. By reducing wind speed, it reduces the evaporation of water from the soil surface, conserves water in the soil by reducing capillary rise, maintains a uniform snow cover and prevents wind erosion of the soil, etc. Based on studies, forest curtains, although they occupy only 3-4% of the land, their presence can increase agricultural production by more than 35%. By creating a network of forest curtains, with an arrangement perpendicular to the prevailing wind direction, we found a 25-50% reduction in wind speed, a significant reduction in evapotranspiration and implicitly the conservation of water in the soil (Andreu et al., 2008; Mize et al., 2008). A well-established forest curtain reduces a small portion of agricultural land, and its benefits far outweigh the loss of productive land. It can be 15-20 m wide, with a 1 m shrub belt planted on both sides (Constandache et al., 2012; Chendev et al., 2015). The first field protection curtains date back to 1696, in southern Ukraine, being planted by order of Tsar Peter the Great. In Romania, the need to establish forest curtains was first mentioned by the great agronomist and politician Ion Ionescu de la Brad, in 1866, who established them on his farm in Neamt County, between 1870 and 1872. In 1960, forest curtains protected one million hectares of land in Dobrogea and the Baragan Plain in 1961, 7000 km of forest curtains protected fields and 1.400 km of protected communication routes (Costachescu, 2007). The main objectives were to demonstrate the influence on restoring and maintaining local microclimatic conditions, to improve soil fertility in its research stations, and to deepen research on the effectiveness and importance of forest protection belts. Soil is one of the most important natural resources for the survival and well-being of mankind, being a fragile resource that can easily undergo

degradation processes, so that mankind must take into account the promotion of optimal land. use, maintenance and improvement of soil productivity and conservation of soil resource (Bălan Mihaela et al., 2024).

MATERIALS AND METHODS

The experiment was carried out in the South-East of Romania, in the Bucu area, Ialomita County (Figure 1). The lithological substrate in the Bucu area, which in this case also represents the parent material of the soils, is made up in the upper part of a finer alluvial stratification but with a fairly large thickness (60-70 cm) and variable texture on which the actual soils appeared and evolved. Geographically, the area falls within the Romanian Plain, the Baragan Plain subregion, the Baragan Ialomița relief unit, the Ialomița Meadow relief subunit, being located on the left side of the sector of the Ialomița Meadow corresponding to the Amara Field, in the Strachina Plain, at an altitude of 20-23 m, in the actual Ialomița Meadow (the difference in level from the terrace being 5-7 m).



Figure 1. Bucu, Ialomita County

The research methods used in this study were those practiced according to the ICPA methodology, 1987, vol I, II and III.

Soil analysis

Analysis methods used to determine chemical properties:

- Organic matter (humus): determined volumetrically by the wet oxidation method according to Walkley-Black, in the Gogoaşă modification – STAS 7184/21-82;
- $CaCO_3$ (carbonates): gasometric method using the Scheibler calcimeter, according to SR ISO 10693:1998 (%);
- The nitrogen content was determined indirectly (by calculation) based on the humus content and the degree of base saturation:

$$IN = \text{humus} \times V / 100$$

- Accessible phosphorus (mobile P): according to the Egner-Riehm-Domingo method and dosed colorimetrically with molybdenum blue, according to the Murphy-Riley method (reduction with ascorbic acid);
- Accessible potassium (mobile K): extraction according to the Egner-Riehm-Domingo method and dosage by flame photometry;
- pH: determined potentiometrically, with a combined glass and calomel electrode, in aqueous suspension at a soil/water ratio of 1/2.5 - SR 7184/13-2001;
- Hydrolytic acidity - extraction with sodium acetate at pH 8.2;
- Sum of bases - Kappen Schoffield Chirita method by extraction with 0.05 n hydrochloric acid.

Analysis methods used to determine physical properties:

- pipette method for fractions ≤ 0.002 mm;
- wet sieving method for fractions 0.002 - 0.2 mm and dry sieving method for fractions > 0.2 mm;
- Bulk density (BD): method of metal cylinders of known volume (100 cm^3) at the current soil moisture (g/cm^3);
- Total porosity (PT): by calculation (% volume -% v/v);
- Aeration porosity (PA): by calculation (% volume -% v/v);
- Degree of settlement (GT): by calculation GT = [(PM – PT)/PMN] x 100 (% volume - % v/v), where: PMN – minimum total porosity required, varied depending on the clay content of the respective sample, is calculated with the formula PMN = 45+0.163 A (% volume - % v/v); PT = total porosity (% v/v); A – clay content (% g/g);
- Wilting coefficient (CO): by calculation, by multiplying the hygroscopicity coefficient by 1.5;
- Field water capacity (CC): by estimation based on texture and apparent density, according to MESP, 1987.

RESULTS AND DISCUSSIONS

A soil profile and three pedological surveyes were opened in different soils, which were morphologically and physico-chemically characterized, according to the guide for the field description of the soil profile and specific environmental conditions.

Profile 1 - Calcaric fluvisols (Figure 2; Table 1)
Coordinates: *44579387 - N and 27461041 - E*
Landscape: *meadow*
Use: *arable*
Rock: *fluvial deposits*
Groundwater: *>3.5 m*



Figure 2. Calcaric fluvisol

Morphological characterization

Ao (0-28 cm): sandy loam, light brown (2.5 YR 3/2 when wet and 2.5 YR 5/4 when dry), moderately developed grain structure, small and medium, weakly plastic, weakly adhesive, very frequent thin roots from cultivated vegetation, weak effervescence, gradual transition;

AC (28-52 cm): fine loamy sand, yellowish brown (2.5 Y 3/4 when wet and yellowish brown 2.5 Y 6/4 when dry), very friable, weakly structured, non-plastic, non-adhesive, frequent fine roots, moderate effervescence in the lower half of the horizon, clear transition;

C1 (52-80 cm): coarse, yellowish loamy sand (5 Y 4/3 when wet and 5 Y 5/6 when dry), unstructured, loose, very friable, frequent $CaCO_3$ pseudomycelia, strong effervescence, clear straight passage;

C2 (80-140 cm): fine, light yellowish sand (5 Y 5/3 when wet and 5 Y 6/6 when dry), unstructured, loose, very friable, frequent $CaCO_3$ pseudomycelia, strong effervescence, clear straight passage.

Table 1. Physical and chemical analyses for Calcaric fluvisols, profile 1

Soil horizon	**Ao**	**AC**	**C_1**	**C_2**
Depth (cm)	0-28	28-52	52-80	80-140
Coarse sand (2-0.2 mm)	15.3	20.1	38.9	42.2
Fine sand (0.2-0.02 mm)	30.2	38.2	24.5	28.7
Dust (0.02-0.002 mm)	36.7	30.3	26.4	24.6
Clay (< 0.002 mm)	17.,8	11.4	10.2	4.5
Texture	SS	UF	UG	NF
pH	7.4	7.6	8.3	8.1
Humus content (%)	2.14	1.32	0.48	0.15
Bulk density (g/cm^3)	1.43	1.51	1.54	1.44
Total porosity (%)	49	48	46	48
Degree of compaction (%)	non-compacted	non-compacted	slightly compacted	non-compacted
Base saturation (V%)	96	98	100	100
Nitrogen index (IN)	2.05	1.29	0.48	0.15
Mobile P (ppm)	19	13	11	6
Mobile K (ppm)	137	118	95	81
Wilting coefficient (CO) (%)	7.2	6.1	5.5	4.6
Field capacity (CC) (%)	13.1	11.2	10.1	8.5
Available water capacity (%)	5.9	5.1	4.6	3.8
Total water capacity (%)	34	32	30	33
Humus reserves (t/ha)	85	47.8	21	-

Secondary profile 1 - Calcaric fluvisols (Figure 3)
Coordinates: *44575728 - N and 27455664 - E*
Relief: *meadow*
Use: *arable*
Rock: *fluvial deposits*
Groundwater: *>3.5 m*



Figure 3. Calcaric fluvisols secondary profile 1

Morphological characterization of the secondary profile 1

Ao (0-38 cm): sandy loam, light brown (2.5 Y 4/3 when wet and 2.5 Y 5/4 when dry), moderately developed grain structure, weak biological activity, non-plastic, non-adhesive, frequent thin roots from cultivated vegetation, gradual transition;

AC (38-70 cm): medium loamy sand, yellowish brown (2.5 Y 4/4 when wet and yellowish brown 2.5 Y 6/4 when dry), very friable, unstructured, non-plastic, non-adhesive, frequent coarse pores, frequent fine roots, moderate effervescence, clear straight transition;

C1 (70-110 cm): fine, yellowish loamy sand (5 Y 5/3 when wet and 5 Y 6/6 when dry), unstructured, loose, very friable, frequent $CaCO_3$ pseudomycelia, strong effervescence, clear straight passage;

C2 (> 110 cm): coarse, light yellowish loamy sand (5 Y 5/3 when wet and 5 Y 6/6 when dry), unstructured, loose, very friable, frequent $CaCO_3$ pseudomycelia, strong effervescence, clear straight passage.

The analytical data for survey 1 in the studied area are presented in Table 2.

Table 2. Physical and chemical analyses for calcaric fluvisols, secondary profile 1

Soil horizon	**Ao**	**AC**	**C_1**	**C_2**
Depth (cm)	0-38	38-70	70-110	>110
Coarse sand (2-0.2 mm)	14.5	23.6	22.3	42.7
Fine sand (0.2-0.02 mm)	31.8	33.8	40.2	25.2
Dust (0.02-0.002 mm)	37.2	31.4	28.6	24.6
Clay (< 0.002 mm)	16.5	11.2	8,9	7.5
Texture	SS	UM	UF	UG
pH	7.3	7.6	8.4	7.9
Humus content (%)	2.1	1.22	0.39	0.11
Bulk density (g/cm^3)	1.43	1.45	1.54	1.51
Total porosity (%)	50	48	46	45
Degree of compaction (%)	non-compacted	non-compacted	non-compacted	non-compacted
Base saturation (V%)	95	96	100	100
Content of calcium carbonate (%)	0.9	3.3	6.5	7.1
Nitrogen index (IN)	1.99	1.17	0.39	0.11
P mobil (ppm)	14	10	6	4
K mobil (ppm)	127	119	75	69
Wilting coefficient (CO) (%)	5.8	5.1	4.5	4.0
Field capacity (CC) (%)	10.6	9.3	8.2	7.4
Available water capacity (%)	4.8	4.2	3.4	3.4
Total water capacity (%)	35	33	30	29
Humus reserves (t/ha)	114	56	24	-

Secondary profile 2 Calcaric fluvisols (Figure 4)
Coordinates: *44576182 - N and 27454943 - E*
Relief: *meadow*
Use: *arable*
Rock: *fluvial deposits*
Groundwater: *>3.5 m*



Figure 4. Calcaric fluvisols secondary profile 2

Morphological characterization of the secondary profile 2

Ao (0-34 cm): sandy loam, dark brown (10 YR 3/2 when wet and 10 YR 5/3 when dry), moderately developed grain structure, weak biological activity, weak plastic, weak adhesive, very frequent thin roots originating from cultivated vegetation, weak effervescence, diffuse, wavy passage;

AC (34-66 cm): fine loamy sand, light brown (2.5 Y 4/4 when wet and yellowish brown 2.5 Y 6/4 when dry), very friable, unstructured, non-plastic, non-adhesive, frequent coarse pores, frequent fine roots, moderate effervescence throughout the depth of the horizon, clear straight passage;

C1 (66-98 cm): medium loamy sand, yellowish (2.5 Y 5/3 when wet and 2.5 Y 6/6 when dry), unstructured, very friable, frequent $CaCO_3$ pseudomycelia, strong effervescence, clear straight passage;

C2 (> 98 cm): coarse loamy sand, pale yellow (5 Y 5/2 when wet and 5 Y 6/4 when dry), unstructured, very friable, frequent $CaCO_3$ pseudomycelia, strong effervescence, clear straight passage (Table. 3).

Table 3. Physical and chemical analyses for calcaric fluvisols, secondary profile 2

Soil horizon	**Ao**	**AC**	**C_1**	**C_2**
Depth (cm)	0-34	34-66	66-98	> 98
Coarse sand (2-0.2 mm)	13.2	17.4	17.1	41.7
Fine sand (0.2-0.02 mm)	31.3	42.3	46.7	27.3
Silty (0.02-0.002 mm)	37.6	29.2	26.8	22.5
Clay (< 0.002 mm)	17.9	11,1	9.4	8.5
Texture	SS	UF	UM	UG
pH	7.5	7.8	8.3	8.6
Humus content (%)	1.94	1.32	0.61	0.16
Bulk density (g/cm^3)	1.37	1.35	1.49	1.46
Total porosity (%)	52	50	48	49
Degree of compaction (%)	non-compacted	non-compacted	non-compacted	non-compacted
Base saturation (V%)	94	98	100	100
Nitrogen index (IN)	1.82	1.29	0.61	0.16
P mobil (ppm)	14	11	9	6
K mobil (ppm)	134	127	95	72
Wilting coefficient (CO) (%)	6.9	6.6	5.7	4.8
Field capacity (CC) (%)	12.5	12.0	10.4	8.7
Available water capacity (%)	5.6	6.0	4.7	3.9
Total water capacity (%)	38	37	32	33
Humus reserves (t/ha)	90	57	29	-

Secondary profile 3 Calcaric fluvisols (Figure 5)
Coordinates: *44582140 - N and 27459925 - E*
Relief: *meadow*
Use: *arable*
Rock: *fluvial deposits*
Groundwater: *>3.5 m*



Figure 5. Calcaric fluvisols secondary profile 3

Morphological characterization of the secondary profile 3

Ao (0-26 cm): sandy loam, dark brown (10 YR 3/2 when wet and 10 YR 4/3 when dry), moderately developed grain structure, moderate biological activity, weak plastic, weak adhesive, very frequent thin roots originating from cultivated vegetation, weak effervescence, gradual transition;

AC (26-70 cm): medium sandy loam, light brown (2.5 Y 4/3 when wet and 2.5 Y 5/4 when dry), crumbly, weakly structured, non-plastic, non-adhesive, frequent coarse pores, frequent fine roots, moderate effervescence, clear transition to the underlying horizon;

C1 (70-125 cm): medium loamy sand, light yellow (2.5 Y 5/3 when wet and 2.5 Y 6/4 when dry), unstructured, re-flowing, very friable, frequent pseudomycelia and $CaCO_3$ concretions, strong effervescence, clear straight passage;

C2 (> 125 cm): fine loamy sand, pale yellow (5 Y 5/2 when wet and 5 Y 6/3 when dry), unstructured, re-flowing, very friable, frequent $CaCO_3$ concretions, strong effervescence, clear straight passage.

Analytical data for secondary profile 3 in the studied area are presented in Table 4.

Table 4. Physical and chemical analyses for calcaric fluvisols, secondary profile 3

Soil horizon	**Ao**	**AC**	**C_1**	**C_2**
Depth (cm)	0-26	26-70	70-125	> 125
Coarse Sand (2-0.2 mm)	12.5	1.4	23.9	20.9
Fine Sand (0.2-0.02 mm)	30.9	37.3	39.6	45.6
Dust (0.02-0.002 mm)	38.2	29.5	25.8	24.2
Clay (< 0.002 mm)	18.4	16.8	10,7	9.3
Texture	SS	SM	UM	UF
pH	7.6	7.8	8.5	8.7
Humus content (%)	1.86	1.19	0.44	0.19
Bulk density (g/cm^3)	1.43	1.46	1.52	1.53
Total porosity (%)	50	49	46	46
Degree of compaction (%)	non-compacted	non-compacted	non-compacted	non-compacted
Base saturation (V%)	98	100	100	100
Content of calcium carbonate (%)	0.5	3.1	7.2	8.3
Nitrogen index (IN)	1.82	1.19	0.44	0.19
Mobile P (ppm)	19	15	11	8
Mobile K (ppm)	141	132	107	96
Hygroscopicity coefficient (%)	5.2	4.7	4.1	3.9
Wilting coefficient (%)	7.8	7.0	6.1	-
Field capacity (%)	14.2	12.8	11.2	-
Available water capacity (%)	6.4	5.8	5.1	-
Total water capacity (%)	35	33	30	-
Humus reserves (t/ha)	69	76	37	-

The criteria regarding the establishment of forest species were the classical ones, which also apply for the establishment of tree-wine plantations, with reference to the physical-geographical conditions of the area but also those characteristic of forest species. Taking into account the current pedoclimatic conditions in the studied areas, based on the criteria presented in the M. O. of 14.02.2022, the main forest species recommended for afforestation were

established and presented. In order to establish technical solutions for the realization of forest or forest curtains, it was taken into account the general stationary framing of the analyzed territories and the pedostatic conditions identified in the field.

Afforestation solutions were established on types and subtypes of soils with ecologically similar characteristics, correlating the ecological requirements of the species with the ecological characteristics of the soils, provided that the component species are adapted to the conditions of increased aridity.

The composition or formula of afforestation includes the percentage participation of the species to be introduced in the afforestation of a given land. The afforestation, nominally indicates, by symbols, the component species specifying the proportion of participation of each species. In the case of landscaping (forest curtains or forests), for the protection of the field, the choice of species and their proportion of participation for different types of afforestation. Solutions was carried out according to the bioclimatic framing and current **pedostatic** conditions, taking into account the appropriate Indigenous basic species.

The station sheet for this area was drawn up, according to Table 5.

Table 5. Stationary unit sheet, Bucu area, Ialomita County

1. Unity and form of relief: Meadow 2. Configuration of land: plane3. Slope: <2 % 4. Exposition X 5. Altitude20-23 m 6. Vegetation: segetal herb												
7	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*
Horizon	***Depth (cm)***	***Humus (%)***	***Soil Texture***	***Colour***	***Skeleton content***	***Structure***	***Humidity***	***Compaction***	***pH***	***Effervescence***	***Soluble salts***	***Concretions***
Ao	*0-28*	*2.14*	*Dusty sandy clay*	*Light brown*	*-*	*grainy moderately developed*	*Ue1*	*Non-compacted*	*7.4*	*light*	*-*	*-*
AC	*28-52*	*1.32*	*Sandy clay fine*	*Yellowish brown*	*-*	*Light structured*	*Ue1*	*Non-compacted*	*7.6*	*moderately*	*-*	*-*
C1	*52-80*	*0.48*	*Sandy clay coarse*	*yellowish*	*-*	*unstructured*	*Ue 1-2*	*Non-compacted*	*8.3*	*strong*	*-*	*common*
C2	*80-140*	*0.15*	*Fine sandy*	*Light yellow*	*-*	*unstructured*	*Ue 2*	*Non-compacted*	*8.1*	*strong*	*-*	*common*
20. Parent materials: river deposits				*24. Zonal and local climate: Continental temperate, specific of meadow*						*28. Type and subtipe of soil: Calcaric fluvisols*		
21. Morphological depth: 130 cm				*25. Groundwater: >3,0 m*						*29. Humus of type: mull calcic*		
22. physiological depth: 40 cm				*26. Hydrological and humidity regime; H 1*						*30. Proposal for works:*		
23. Wind erosion: poorly eroded				*27. Character of floods: unenviable*						*- establishement of forest plantation*		
										- energic mobilization of soil		
Stational groupe: GS 14 *Composition of afforestation: 40 St.b (St, Ce, Gr) 30 Aj (Mj, Pă, Ju, Dd) 30 Arb (Lc, Pd, Co, Mc)*												
Observation: Several condition of station (dry-arid climate, carbonate soil, natural regeneration occurs with difficulty												

CONCLUSIONS

● The studied territory belongs to the urban area of Bucu, Ialomita County, geographically located in the Baragan Plain, Mostistea subunit, characterized by a temperate continental climate, specific plain relief, with groundwater at over 3 m.

● The area studied within TC Bucu, Ialomita County, is 55.6325 ha, arable land, from which 24 soil samples were collected and analysed.

● The purpose of the work was to know the properties of the soil in order to use it judiciously, determine the quality class and suitability of the land for forest species.

● The soil cover in the studied area is consistent with the physical-geographical conditions of the area, with only one type of soil with a local character being identified (calcaric fluvisols).

● The parent material is predominantly composed of fluvial deposits.

● The texture of this soil unit is generally coarse, with contrasting distribution (sandy clay in the bioaccumulative horizon, loamy sand in the transition horizon and coarse towards the base of the soil profile).

● The main limiting factor of production potential is the poor rainfall during the growing season, combined with the less favorable physical properties of the soil.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

SUSTAINABLE USE AND MANAGEMENT OF SOIL RESOURCES IN THE ALMĂJULUI VALLEY AREA, COUNTY CARAȘ-SEVERIN

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Abstract

The proposed scientific paper focuses on "Sustainable use and management of soil resources in the Almăjului Valley area, County Caraș-Severin". It will look at the importance of soils as essential resources for agriculture, biodiversity, sustainability and environmental sustainability. The study will highlight the current practices of agricultural land use (arable, pasture, hayland, trees and vineyards) and the impact of mainly agricultural activities on their quality. In addition, it will examine sustainable management/stewardship strategies that can be implemented to prevent degradation, soil pollution to promote an increase in fertility in the medium and long term. The paper will propose specific and concrete measures to protect and prevent soil clearance, such as crop rotation, use of organic fertilisers according to legal management requirements (SMR) and standards on good agricultural and environmental conditions of land (GAEC), rational grazing, application of agroforestry systems and ecosystem restoration, thus also highlighting the role of local communities in the implementation of these sustainable and sustainable practices.

Key words*: soil, use, management, sustainable, resources, fertilization.*

INTRODUCTION

Sustainable management of soil resources is essential for maintaining healthy ecosystems, biodiversity and human well-being. Soil is a complex environment that supports agriculture, regulates biogeochemical cycles, stores water and carbon, and serves as a habitat for numerous organisms. The importance and necessity of sustainable soil management derives from the fact that it is a finite resource, vulnerable to degradation due to anthropogenic activities, climate change and economic pressures (Țărău et al., 2019; Niță et al., 2018; David et al., 2018).
A fundamental aspect of sustainable soil management is protecting soil quality. Intensive agriculture through the excessive use of pesticides and chemical fertilizers, deforestation and accelerated urbanization contribute to soil degradation, which can lead to degradation processes such as erosion, compaction, decreased fertility and contamination. By implementing sustainable practices, such as crop rotation, agroforestry systems, soil cover with vegetation, the use of organic fertilizers and moisture conservation, the structure can be improved, and thus agricultural production will be optimized in the long term (Montgomery, 2007; Blaga et al., 2008).
Sustainable soil management plays a crucial role in combating climate change, as soils function as a carbon sink, and proper soil management can help reduce greenhouse gas emissions. Sustainable farming practices not only help to store carbon, but also improve the soil's ability to retain water, which is essential in the context of extreme weather events such as droughts or floods.
Last but not least, the sustainable management of soil resources has a significant social and economic impact because healthy soil is essential for ensuring food security, sustaining local economies and protecting vulnerable communities, so by increasing agricultural productivity and reducing the risks posed by soil degradation processes, favorable conditions can be created for the sustainable development of communities (Quinton et al., 2010).
The sustainable management of soil resources is vital for ensuring sustainable economic development, protecting the environment and promoting social well-being, thus becoming a global priority. The aspects regarding the use of information regarding the soil resources in the

studied area are accumulated from the pedological studies, stored in the archive of the specialized regional institutions, (mostly on classical support, but also on the basis of SPED 1, BDUST information systems) and part of the information and data are taken from the research programs carried out over time by the authors (within USV Timișoara), for the qualitative and quantitative evaluation of the ecopedological resources in the Almăjului Valley and of the possible pressures on them, but also on measures to promote environmentally friendly social practices (Methodology for the elaboration of pedological studies, 1987).

MATERIALS AND METHODS

The soil resources in the analyzed area extend over a total area of 109,518 hectares (Table 1), of which 40,628 hectares (37.11%) are used as agricultural land, and 60,323 hectares (60.33%) are covered by forest vegetation. These lands are located in the Almăjului Valley and are administered by the seven administrative-territorial units: Bănia, Bozovici, Dalboșeț, Eftimie Murgu, Lăpușnicu Mare, Prigor and Șopotu Nou, all located in Caraș-Severin County (Țărău et al., 2019; Mircov et al., 2021). Analyzing the land use in the depression of the Almăjului Valley, it is highlighted that significant areas are dedicated to pastures and meadows, which constitute approximately 27.5% of the total area and over 74% of agricultural land. In contrast, the area under arable cultivation represents only 21.77% of the agricultural land (Table 1). The area of the Almăjului depression is distinguished by a long tradition in agriculture, this activity having a considerable impact on the economy.

Table 1. Situation of the land fund in Almăjului Valley (Niță et al., 2024)

Crt. No.	Locality	Arable	Pasture	Grassland	Vignards	Orchards	Total agricultural	Forests	Waters	Other categories	Total general
1	Bănia	1720	4566	853	0	377	7516	12437	75	564	20592
2	Bozovici	1177	4300	1514	0	323	7314	11486	69	710	19579
3	Dalboșeț	1224	2674	1201	0	140	5239	3061	61	266	8627
4	Eftime Murgu	994	1706	478	0	315	3493	6103	63	211	9830
5	Lăpușnicu Mare	707	3281	1444	0	222	5654	6446	57	209	12366
6	Prigor	2089	3591	1714	0	291	7685	22075	60	367	30187
7	Șopotu Nou	935	2058	694	0	40	3727	4495	12	103	8337
Total (ha)		**8846**	**22176**	**7898**	**0**	**1708**	**40628**	**66103**	**397**	**2430**	**109518**
% total area		**8.08**	**20.25**	**7.22**	**0**	**1.56**	**37.11**	**60.33**	**0.36**	**2.20**	**100**
% total agricultural		**21.77**	**54.58**	**19.44**	**0**	**4.21**	**100**	**-**	**-**	**-**	**-**

The research of the ecopedological conditions of formation and evolution of soil resources was carried out according to the national standards represented by the Methodology for the elaboration of pedological studies (vol. I, II, III), developed and created by ICPA Bucharest in 1987. This methodological standard was completed with specific aspects of the Romanian Soil Taxonomy System (SRTS-2012) (Florea & Munteanu, 2012), MADR Order 278/2011 - National Program on the Implementation of the National Soil-Field Monitoring System for Agriculture. The study was based on the pedological data available in the OSPA archive in Timișoara, which contains information from over 68 years, as well as on previous research carried out by various authors, supplemented with recent data obtained from the field by the authors.

RESULTS AND DISCUSSIONS

The factors that limit the production capacity of soils in the Almăjului Valley include physical features such as the degree of compaction, the slope of the land, the erosion process, the excess of groundwater moisture and rainfall. Also, agrochemical properties, such as soil reaction, humus content and levels of other essential nutrients (such as phosphorus and potassium), play a decisive role in this production capacity. The degree of compaction refers to the densification of soil particles, which leads to excessive compaction.

This can limit the circulation of air and water in the soil, thus affecting root development and nutrient uptake by plants. Compacted soils have a reduced capacity to retain moisture and can inhibit the activity of microbial organisms, which are essential for the process of decomposition of organic matter and nutrient cycling. Compaction can also lead to increased erosion by reducing the stability of soil structures.

The slope of the land greatly influences the erosion process and water runoff. On steeper slopes, rainwater accumulates and flows faster, increasing the risk of surface erosion and transport of fertile soil. This can lead to the loss of the top layer of soil, which is the richest in nutrients. Additionally, steep slopes can also affect water infiltration capacity and lead to landslides.

Soil erosion refers to the process of soil degradation caused by external agents such as water and wind. Erosion can remove the top layer of soil, which contains most of the nutrients needed by plants. Erosion can be accelerated by anthropogenic activities such as intensive agriculture, deforestation, and road construction. This not only reduces soil fertility, but also contributes to the pollution of water sources by transporting sediments and chemicals.

Excess groundwater refers to the level of groundwater. An excess of this moisture can create anaerobic conditions in the soil, preventing oxygen from reaching the roots, which can lead to root apoplexy and root rot. It can also influence the availability of nutrients, as certain nutrients become more soluble and can be washed out of the soil, reducing its fertility.

Excess rainfall moisture can lead to soil saturation, which generates anaerobic conditions similar to those caused by groundwater. Additionally, torrential rains can create excessive runoff and contribute to erosion. This excess moisture can affect not only the roots of plants, but also the activity of beneficial organisms in the soil, thus reducing its ability to sustain agriculture.

Soil reaction (pH is a crucial indicator of soil health and influences the availability of nutrients to plants. An optimal pH for most agronomic plants is between 6.0 and 7.5. Acidic soils (pH < 6.0) can limit the availability of essential nutrients such as phosphorus, thus reducing soil fertility. In alkaline soils (pH > 7.5), deficiencies in microelements such as iron and zinc may occur. Also, the reaction of the soil affects the activity of beneficial microorganisms and the processes of mineralization of organic matter.

Humus is the stable part of organic matter in the soil and is essential for its fertility. Humus content influences water retention capacity, soil structure, and microbiological activity. A high humus content improves the soil's ability to retain nutrients and water, creating a favorable environment for plant development. Humus also contributes to soil stabilization and erosion prevention. Decreasing humus content, often due to intensive farming practices, can lead to reduced fertility and deterioration of soil structure.

Levels of nutrients such as phosphorus (P) and potassium (K) are essential for healthy plant development. Phosphorus plays a crucial role in the processes of photosynthesis, respiration and root development, being a vital element in the synthesis of DNA and ATP (adenosine triphosphate). A phosphorus deficiency can lead to slow plant growth and poor development of the root system. Potassium, on the other hand, is essential for regulating osmosis and water balance, contributing to the overall health of the plant, including resistance to water stress and disease. If these two nutrients are not available in sufficient quantities, soil fertility is severely affected and agricultural production can suffer.

All these limiting factors interact in a complex way and can influence soil fertility, thus impacting agricultural production and ecosystem health. Sustainable management of these conditions is crucial for maintaining adequate soil fertility.

In the administrative-territorial unit of Bănia, the limiting factors that significantly affect the quality of the soil are determined by the following aspects: a high degree of compaction, which affects approximately 49.8% of the area; the slope of the land and the risk of erosion, which is manifested on 49.5% of the territory; excess groundwater, present on 28.6% of the surface; excess rainfall, observed on 67.9% of the area; and moisture from overflows, which affects 18.9% of the land. In terms of agrochemical characteristics, the soil reaction is

moderately and strongly acidic in proportion of 77.8%. There is also a low content of humus and other essential nutrients, such as nitrogen, phosphorus and potassium, which affect 62.9% of the total area.

In Bozovici, the limiting factors influencing the quality of the soil cover are the following: soil acidity, low level of humus reserve, texture, high compactness (characterized by very low porosity), soil stability, slope, landslides, soil unevenness, surface erosion (including the risk of erosion) and excess moisture (groundwater, surface, from overflows or runoff on slopes). Other aspects include low temperature and moisture deficit.

Soil acidity is a limiting factor on approximately 44.38% of the analyzed area, with low pH values, with the following categories of limitations: moderate on 23.11% and reduced on 21.27%.

The humus reserve, due to its constituent and dynamic features, presents severe limitations on 0.85% of the territory, moderate on 59.16% and reduced on 22.17%.

As for compactness, it has severe limits on 0.50%, moderate on 9.40% and reduced on 0.18%.

The reduced edaphic volume is signaled with the following types of limitations: extremely severe on 0.38%, very severe on 33.28%, severe on 14.40%, moderate on 3.65% and reduced on 4.64%.

Landslides are classified according to severity, with extremely severe limitations on 3.31%, moderate on 1.29% and reduced on 7.05%. These landslides favor the appearance of excess moisture on the slopes, with moderate limitations on 9.98% and reduced on 14.21%.

Excess surface moisture is characterized by severe limitations on 0.93%, moderate on 1.06% and reduced on 0.17%.

In Dalboșeț Commune, the limiting factors that have a significant impact on soil quality include the following aspects: Deficit of the humus reserve, which is moderately manifested on 42% of the surface, severe on 3.8% and very severe on 15%. Humus moisture is essential for soil fertility and health. The acidity of the soil, with moderate limits on 21%, affects the availability of nutrients and biological activity in the soil. The fine soil texture is associated with moderate limitations on 0.5%, and the coarse texture has severe limitations on 2.8%. The texture of the soil influences its ability to retain moisture and aerate. The useful edaphic volume has severe limitations on 38% of the territory and very severe on 1%. This affects the soil's ability to support vegetation and, implicitly, agricultural production. The slope of the land contributes severe limitations on 48% and very severe on 15%, which can lead to erosion and difficulties in water management. Landslides are characterized by extremely severe limitations on 0.32%, affecting soil stability and infrastructure. Excess groundwater moisture is present with severe limitations on 4.2% and very severe on 3.7%, which can cause drainage problems and affect plant roots. The excess stagnant moisture manifests itself moderately on 10.7% of the territory and severely on 4.2%, and the floodability presents severe limitations on 3.6%, which can complicate the cultivation of plants and affect the quality of the soil as a whole.

Within the administrative-territorial unit Eftimie Murgu, the limiting factors that affect the quality of the soil at the level of the cadastral territory are the following: The soil reaction is characterized by low values, being classified as follows: 32.10% slightly acidic soils, 26.70% moderately acidic and 29.90% strongly acidic. This acidity can influence the availability of essential nutrients for vegetation and the activity of microorganisms in the soil. The excess of surface moisture manifests itself variablely, with low levels on 18.60% of the land, moderate on 14.50% and strong on 32.87%. This excessive accumulation of water can lead to stagnation, affecting plant roots and contributing to erosion. Excess groundwater moisture is present in varying degrees, with low humidity at 8.80%, moderate at 11.60%, strong at 7.70%, and excessive at 3.09%. This factor can favor anaerobic conditions, affecting plant development and contributing to soil degradation. The bearing capacity of the land is assessed as temporarily unsatisfactory on 24.05% of the surface, poor on 15.50% and moderate on 12.60%. It affects the soil's ability to support structures and vegetation, thus influencing agricultural production. The slope of the land varies between 5.1% and 35.0% and is over 45.1% on 45.70% of the territory. Steep slopes can increase the risk of erosion and water runoff, affecting soil stability and fertility.

In the Commune of Lăpușnicu Mare, the limiting factors that affect the quality of the soil cover are the following: The reaction of the soil, with acidified values, affects 43.8% of the surface. This acidity can significantly influence the availability of essential nutrients and the activity of beneficial microorganisms in the soil. The low reserve of humus is manifested on 36.3% of the territory, which can reduce the soil's ability to retain water and nutrients, essential for the growth of vegetation. Soil compactness is a major limiting factor, affecting 57.6% of the surface. Excessive compaction can restrict the circulation of air and water in the soil, thus compromising the healthy development of plant roots. The slope of the land contributes to the limitations of the soil, with extremely severe evaluations on 17.3%, very severe on 12.2% and severe on 21.4%. Steep slopes can increase the risk of erosion and affect soil stability, negatively influencing soil fertility. Excess groundwater is another limiting factor, with severe limitations on 6.6% and moderate limitations on 3.6%. This excess groundwater can generate anaerobic conditions, affecting the health of plant roots. Excess stagnant moisture is observed on 8.6% of the land, which can lead to stagnation of water on the surface and deterioration of soil quality, negatively affecting vegetation growth.

In Prigor Commune, the limiting factors that affect the quality of the soil cover within the cadastral territory are the following: The soil reaction is predominantly acidic, with low values: 12.20% of the soils are weakly acidic, 6.20% moderately acidic and 73.85% strongly acidic. This high acidity can negatively influence the availability of nutrients and the activity of beneficial microbial organisms in the soil. The excess of surface humidity is variable, having a low manifestation on 28.30% of the territory, moderate on 12.60% and strong on 19.60%. Excessive accumulation of water on the surface can lead to stagnation, affecting plant root health and the potential for erosion. Excess groundwater is observed with limitations: low on 8.80%, moderate on 10.20%, strong on 9.60% and excessive on 1.95%. This phenomenon can create anaerobic conditions in the soil, affecting root development and nutrient availability. The bearing capacity of the land is assessed as temporarily unsatisfactory on 37.60% of the surface, poor on 12.30% and moderate on 16.20%, which affects the soil's ability to support vegetation and infrastructure. The slope of the land varies, with values between 5.1% and 35.0% and over 45.1% affecting 55.20% of the territory. Steep slopes can intensify erosion and water runoff, influencing not only soil fertility but also its stability.

In Șopotu Nou Commune, the limiting factors influencing the quality of the soil cover within the cadastral territory are the following: The soil reaction presents low values, being distributed as follows: 25.50% of the soils are weakly acidic, 26.80% are moderately acidic, and 32.90% are strongly acidic. This variable acidity can affect the availability of nutrients to plants and the efficiency of biological processes in the soil. Excess surface moisture manifests itself variably, with 8.60% of the surface having low humidity, 6.80% moderate and 9.30% strong. Excessive accumulation of water on the surface can lead to stagnation of grasslands and compromise plant health.The bearing capacity of the land is assessed as temporarily unsatisfactory on 8.20% of the land, poor on 10.10% and moderate on 6.50%. These assessments influence the soil's ability to support vegetation and built structures. The slope of the land has varied values, with over 65.10% of the surface having slopes ranging from 5.1% to 35.0% and the steepest ones, with values higher than 45.1%. Higher slopes can increase the risk of erosion and affect soil stability, negatively impacting fertility.

These factors interact complexly, influencing not only soil fertility, but also its use for agricultural activities and the health of the local ecosystem.

CONCLUSIONS

This paper reveals an extensive research on the sustainable use and management of soil resources in the Almăjului Valley, Caraș-Severin County. The study highlights the importance of soil as an essential resource for agriculture, biodiversity and environmental sustainability. The research uses a rigorous methodology, based on national and international standards, integrating historical data from the O.S.P.A. Timișoara archive with

recent data from the field. The conclusions focus on identifying the limiting factors of soil fertility in different administrative-territorial units in the area.

The study identifies a series of limiting factors that affect the production capacity of soils in the Almăjului Valley. These factors are grouped into physical, physico-mechanical, chemical and biological categories.

Physical factors include the degree of compaction (compaction), the slope of the land, erosion, excess moisture (groundwater, rainwater, overflows). Compaction reduces soil permeability, affecting aeration and water infiltration, which inhibits root development and microbial activity. Steep slopes intensify erosion, loss of fertile layer and can lead to landslides. Excess moisture, regardless of the source, creates anaerobic conditions that limit root development and nutrient availability.

Among the agrochemical properties, acidity (low pH), low humus content and deficiencies of essential nutrients (nitrogen, phosphorus, potassium) stand out. The acidity of the soil affects the availability of nutrients, the activity of microorganisms and mineralization processes. Low humus levels reduce the capacity to retain water and nutrients, affecting soil structure and fertility. A deficiency of essential nutrients limits plant growth and development.

The paper draws attention to the impact of anthropogenic activities, especially intensive agriculture, on soil degradation. Excessive and uncontrolled use of pesticides and chemical fertilizers, deforestation, and accelerated urbanization contribute to erosion, compaction, decreased fertility, and contamination.

The study emphasizes the spatial variability of limiting factors, with significant differences between the administrative-territorial units analyzed. For example, in Bănia, compaction and excess rainfall are major problems, while in Bozovici, acidity, low humus reserve and compactness are main limiting factors. This variability requires management approaches tailored to the specific conditions of each area.

From the point of view of sustainable management strategies, the research proposes the implementation of sustainable soil management strategies to combat degradation and promote long-term fertility growth. These strategies include: crop rotation, agroforestry systems, vegetation cover, use of organic fertilizers, moisture conservation, rational grazing practices, and ecosystem restoration. The involvement of local communities is essential in the implementation of these strategies.

This paper aims to present a complex and detailed assessment of the limiting factors of soil fertility in the Almăjului Valley, highlighting the spatial variability of these factors and emphasizing the need to implement integrated strategies for sustainable soil management.

Combating soil degradation and promoting sustainable agriculture are essential to ensure food security, environmental protection and sustainable economic development of the region.

The conclusions of the research should serve as a basis for the development of effective policies and strategies for the conservation and sustainable use of soil resources in the Almăjului Valley and other regions with similar characteristics.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

SOIL NUTRITIONAL STATUS FUNCTION OF BIOMASS HARVESTING IN NORTHWESTERN ROMANIA

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Abstract

The removal of biomass disrupts the natural nutrient cycle in forest ecosystems. Organic materials like leaves and branches, which would typically decompose on the forest soil, are essential for replenishing soil nutrients. Key nutrients such as nitrogen, phosphorus, and potassium are particularly affected. These nutrients are vital for plant growth and soil health, and their depletion can lead to reduced soil fertility over time. These nutrients are crucial for maintaining soil health and supporting plant growth. The present study carried out in forest area of Cluj County, Northwestern Romania, during summer and autumn of 2024, explores the relationship between biomass harvesting practices and the nutritional status of soil in forest ecosystems. Raw data collected from field were statistically processed using XLSTAT program. The results of our research show that removal of biomass, including branches, leaves, and other organic materials, can significantly impact soil fertility by depleting essential nutrients such as nitrogen, phosphorus, and potassium.

Key words*: deforestation, forests ecosystem, nutrients, plants growth.*

INTRODUCTION

Biomass harvesting, whether from forests or agricultural systems, plays a pivotal role in the renewable energy sector and sustainable resource management (Bays et al., 2024; Miner et al., 2014; Titus et al., 2021). Meantime, it is well known that biomass removal (especially whole tree harvesting) has detrimental effect on soil productivity. The depletion of soil nutritional satus due to biomass harvesting are more pronounced in the forest floor than in the mineral soil. In the initial years following harvest, biomass removal can significantly impact environment and soil ecosystems by modifying microclimatic conditions and nutritional status (Agbeshie et al., 2025; Bellassen & Luyssaert, 2014; di Maria, 2025; Veldkamp et al., 2020). Over time, reduced nitrogen and/or phosphorus and potassium availability in whole-tree harvested sites has been associated with diminished soil fertility, persisting for at least 20 years in some forest stands (Akselsson & Westling, 2005; Duchesne & Houle, 2008; Thiffault et al., 2011). In North-West Romania, extensive forest biomass removal, mainly whole tree, has become a common practice, driven by both energy needs and the economic benefits associated with biomass-based industries (Andronache et al., 2017; Pintilii et al., 2015). Yet, these practices raise critical questions about the long-term health of the soil. Soil nutritional status is maintained through a continuous cycle of organic matter deposition and microbial decomposition (Dinesh et al., 2003; Khresat et al., 2007). Nitrogen is vital for protein synthesis and plant growth, phosphorus is essential for energy transfer within cells, and potassium regulates enzyme activation and water use efficiency (Kassa et al., 2017). In intact ecosystems, these nutrients are naturally replenished through plant residues, animal activity, and microbial processes. Changes in land use influence stream nutrient chemistry by altering nutrient cycling within vegetation and soil organic matter (Biggs et al., 2004). Biomass removal interrupts this cycle. The loss of organic inputs can lead to a decline in soil organic matter, thereby reducing the soil's capacity to retain and recycle essential nutrients (Biggs et al., 2004; Kučerík et al., 2018). The clearing of forested land may have negative impact on physical and nutritional soil properties, leading to the degradation of nitrogen, when compared to land under natural forests, reforestation, or

grassland (An et al., 2008). The study was conducted to analyze the impact of biomass removing on soil physical and nutritional properties by comparing forested areas with those where biomass was removed, deforested, respectively.

MATERIALS AND METHODS

The trial was carried out in forest area of Cluj County, located in Northwestern Romania, populated with deciduous species, during summer and autumn of 2024. According to ISRIC - International Soil Reference and Information Centre (https://www.isric.org/), the soil belongs to Luvisol group. Soil samples were collected from 10 points located in both environments, forested and deforested. Deforested area is the result of whole tree harvesting which was made during 6-8 years ago. In each area, samples were taken from 0- 30 cm depth from the horizon Ao in each soil. The collected soil samples were analyzed for physical parameters: clay, sand, and dust fractions, total porosity, and apparent density. The analyzed soil nutritional properties concern pH, humus content, nitrogen, phosphorus, and potassium levels. Laboratory analyses were conducted using standardized methods. Particle size distribution was determined using the hydrometer method, while total porosity and apparent density were measured through gravimetric techniques. Soil pH was analyzed using a pH meter, and humus content was assessed through wet oxidation. Nitrogen, phosphorus, and potassium levels were determined using Kjeldahl, spectrophotometric, and flame photometry methods, respectively. Climatic variables such as temperature, relative humidity, wind velocity, and precipitation were monitored in the field using a mobile station. Statistical analyses, including descriptive statistics, cluster analysis and PCA, were performed using XLSTAT and Statistica for windows v.18.0 software.

RESULTS AND DISCUSSIONS

The comparative analysis of the physical characteristics of soil in forested and deforested areas shows the impact of biomass removing on soil structure and composition (Table 1).

Table 1. The physical traits of soil located in forested and deforested areas

Trait	N	X	Min.	Max.	s	CV, %
Forested area						
Raw sand (%)	10	0.32a	0.30	0.33	0.01	3.61
Fine sand (%)	10	2.76a	2.50	3.00	0.21	7.46
Dust I (%)	10	12.49a	12.00	12.90	0.39	3.09
Dust II (%)	10	21.27a	20.00	22.00	0.80	3.78
Clay (%)	10	34.92a	34.00	35.70	0.62	1.76
Hygroscopicity (%)	10	5.68a	5.00	6.20	0.47	8.26
Apparent density (g/cm^3)	10	1.26a	1.00	1.90	0.37	28.88
Total porosity (%)	10	51.30a	49.00	53.00	1.72	3.35
Deforested area						
Raw sand (%)	10	0.55b	0.52	0.57	0.02	3.64
Fine sand (%)	10	2.21a	2.00	2.40	0.16	7.45
Dust I (%)	10	8.62b	8.28	8.90	0.27	3.08
Dust II (%)	10	9.03b	7.80	10.43	0.95	10.55
Clay (%)	10	25.15b	24.48	25.70	0.44	1.76
Hygroscopicity (%)	10	4.41a	3.60	4.96	0.50	11.30
Bulk density (g/cm^3)	10	1.73a	1.37	2.60	0.50	28.88
Total porosity (%)	10	35.91b	34.30	37.10	1.20	3.35

In the forested area the soil has a higher clay content and total porosity (34.92%, and 51.30%, respectively) which contribute to better water retention and aeration.

The apparent density is lower, indicating a more stable and less compacted soil structure. In the deforested area there is an increase in raw sand content (0.55% compared with 0.32 reported in forested area) and a decrease in clay and dust fractions, suggesting soil degradation and erosion. The total porosity is lower while apparent density is higher indicating compaction and reduced water infiltration. The differences between forested and deforested areas are significant for clay content, total porosity and dust fractions. The deforested area shows a marked decline in fine particles that suggest the reducing of its ability to retain moisture and support plant growth.

Similar results concerning clay content are revealed by Hajabbasi et al. (1997) in Iran forest areas, who report a statistically significant decrease from 37.9% in forested soil to 28.6% in deforested soil.

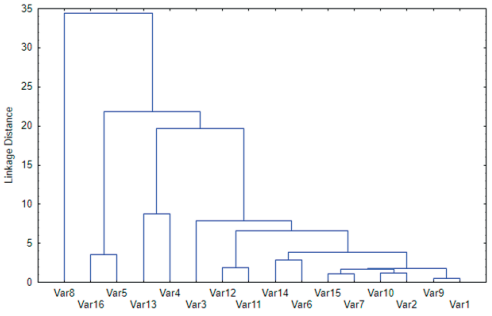
If analyzing the nutritional properties of soil in forested and deforested areas, one may find the biomass removing impacts on soil fertility. In the forested area the pH is higher (6.44 pH units), humus content (1.63%) is more abundant and nitrogen phosphorus and potassium levels (0.13%, 11.94 ppm, and 91.80 ppm, respectively) are significantly greater, indicating a more fertile soil environment capable of supporting plant growth and microbial activity. Similar results concerning nitrogen content are presented by Hajabbasi et al. (1997) in Iran forest areas, who report a statistically significant decrease from 0.164% in forested soil to 0.009% in deforested soil.

In the deforested area there is a notable reduction in humus (1.04%), nitrogen (0.08%), phosphorus (540 ppm) and potassium (49.91 ppm) levels, which suggests depletion of organic matter and essential nutrients. The high variability in phosphorus and nitrogen indicates instability, likely caused by soil erosion and loss of nutrient retention capacity. The pH is slightly lower pointing to potential acidification after biomass removing. The decrease in soil fertility in deforested areas highlights the negative consequences of vegetation removal, reducing the ability of the soil to sustain plant growth and increasing the need for restoration measures to replenish organic matter and essential nutrients (Table 2).

Table 2. The nutritional traits of soil located in forested and deforested areas

Trait	N	X	Min.	Max.	s	CV, %
Forested area						
pH (pH units)	10	6.44a	6.30	6.60	0.11	1.75
Humus (%)	10	1.63a	1.45	1.80	0.16	9.94
Nitrogen (%)	10	0.13a	0.12	0.14	0.01	6.57
Phosphorus (ppm)	10	11.94a	11.00	13.00	0.75	6.31
Potassium (ppm)	10	91.80a	86.00	99.00	5.17	5.63
Deforested area						
pH (pH units)	10	6.13a	6.05	6.24	0.08	1.34
Humus (%)	10	1.04b	0.80	1.26	0.16	15.79
Nitrogen (%)	10	0.08b	0.06	0.08	0.01	13.77
Phosphorus (ppm)	10	5.40b	0.65	7.02	0.70	12.96
Potassium (ppm)	10	49.91b	43.00	53.46	4.57	9.16

The clustering pattern indicates that total porosity in both forested and deforested areas (Var8 and Var16) are represented in distinct groups, suggesting a significant difference in porosity between the two environments. Clay in deforested and forested areas (Var5 and Var13) groups are closely positioned, highlighting their similarity despite environmental changes. Dust I and dust II in both areas (Var3, Var4, Var11, Var12) exhibit related clustering suggesting that these fractions behave similarly under both conditions. Traits related to apparent density (Var7 and Var15) and hygroscopicity (Var6 and Var14) also cluster closely suggesting a relationship between these properties in maintaining soil structure and moisture retention. The raw sand, and fine sand (Var1, Var2, Var9, Var10) are grouped together at lower linkage distances indicating minimal variation in their distribution across forested and deforested soils (Figure 1).

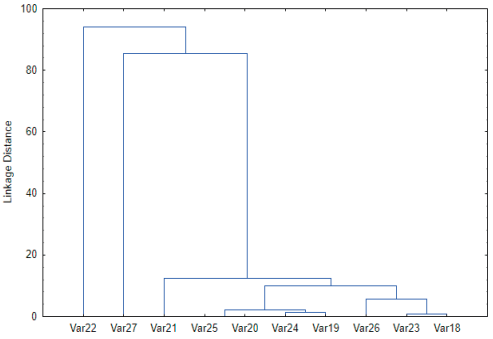


Var1 - raw sand, forested; Var2 - fine sand, forested; Var3 - dust I, forested; Var4 - dust II, forested; Var5 - clay, forested; Var6 -hygroscopicity, forested; Var7 - apparent density, forested; Var8 - total porosity forested; Var9 - raw sand, deforested; Var10 - fine sand, deforested; Var11 - dust I, deforested; Var12 - dust II, deforested; Var13 -clay, deforested; Var14 - hygroscopicity, deforested; Var15 - bulk density, deforested; Var16 - total porosity deforested.

Figure 1. The dendrogram corresponding to physical traits of soil located in forested and deforested areas

Concerning the soil nutritional traits, the cluster analysis shows that the most distinct grouping is observed for potassium in both forested and deforested soils (Var22 and Var27), which cluster at the highest linkage distance. This

suggests a strong differentiation in potassium availability between the two environments.
Phosphorus in both conditions (Var21 and Var26) forms a separate cluster at a significant distance, indicating differences in phosphorus levels due to biomass removing (Figure 2).



Var18 - pH, forested; Var19 - humus, forested; Var20 - nitrogen, forested; Var21 - phosphorus, forested; Var22 - potassium, forested; Var23 - pH, deforested; Var24 - humus deforested; Var25 - nitrogen, deforested; Var26 - phosphorus, deforested; Var27 - potassium deforested.

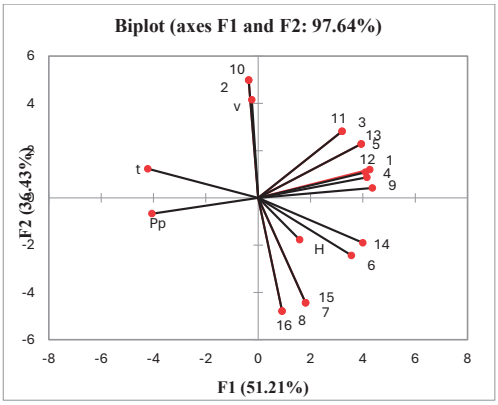
Figure 2. The dendrogram corresponding to nutritional traits of soil located in forested and deforested areas

Nitrogen in forested and deforested areas (Var20 and Var25) correspond to separate clusters. Humus (Var19 and Var24) and pH (Var18 and Var23) exhibit the smallest linkage distances suggesting that these traits remain relatively stable between forested and deforested conditions compared to other nutrients. The clustering pattern indicates that potassium, phosphorus nitrogen and humus soil contents changed due to biomass removing, while pH shows relatively smaller variations, thus reinforcing the importance of these elements in assessing soil fertility degradation and the need for nutrient restoration in deforested areas.
PCA was conducted, considering the results of the Bartlett ($p<0.001$) and Keiser - Meyer - Olkin (KMO) tests (Merce & Merce, 2009). We identified three principal factors affecting soil physical and nutritional traits, in both forestation and biomass removing conditions. These factors are: Factor 1, environmental conditions (forestation and biomass removal), Factor 2, climatic conditions, and Factor 3, soil status (structural and nutritional). According to PCA, Factor 1 - Environmental conditions explains 56.20% of the total variance indicating that it captures most of the information concerning the soil physical traits in both analyzed environmental conditions, forestation and biomass removing, respectively. Factor 2 - Climatic conditions, accounts for 41.43% bringing the cumulative variance explained to 97.64%, which suggests that these two factors are determinant for the soil physical traits. Factor 3 contributes only 2.36% to the total variance, indicating minimal additional information beyond the first two factors and confirming that most of the variability in the data is well represented by the first two principal components (Table 3).

Table 3. The Eigenvalues and total variance for physical traits of soil located in forested and deforested areas

Eigenvalue	% Total - variance	Cumulative - Eigenvalue	Cumulative - %
11.2417	56.2087	11.2417	56.2087
8.2859	41.4324	19.5276	41.4324
0.4724	2.3600	20.0000	100.0000

Soil variables such as total porosity (8 and 16) and apparent density (7 and 15) exhibit strong contributions along PC1 suggesting significant differentiation between forested and deforested areas. The clay (5 and 13) and dust fractions (3, 4, 11, 12) align in a way that highlights their role in soil composition and aggregation. Environmental factors, temperature (t) relative humidity (H) wind velocity (v) and precipitation (Pp), respectively, show distinct orientations indicating their independent influence on soil properties (Figure 3).



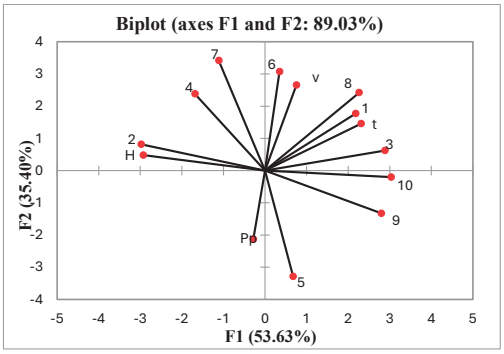
1-raw sand, forested; 2-fine sand, forested; 3-dust I, forested; 4-dust II, forested; 5-clay, forested; 6-hygroscopicity, forested; 7-bulk density, forested; 8-total porosity forested; 9-raw sand, deforested; 10-fine sand, deforested; 11-dust I, deforested; 12-dust II, deforested; 13-clay, deforested; 14-hygroscopicity, deforested; 15- bulk density, deforested; 16-total porosity deforested; t-temperature; H-relative humidity; v-wind velocity; Pp-precipitations. The arrow represents the direction of the weighting of soil analyzed traits.

Figure 3. The representation in PC1 x PC2 plans of the variables corresponding to physical traits of soil located in forested and deforested areas

The clear separation of soil traits between forested and deforested areas underscores the impact of biomass removing on soil structure porosity and density while climatic factors play a role in shaping these physical characteristics.
Similarly with results concerning the physical soil trats, when analyzing nutritional soil status, PCA shows that most of the variability in the data is well represented by the first two principal factors. Factor 1 explains 53.63% of the total variance and Factor 2 accounts for 35.40% (Figure 4). Factor 3 makes a 10.97% contribution to the total variance (Table 4).

Table 4. The Eigenvalues and total variance for chemical traits of soil located in forested and deforested areas

Eigenvalue	% Total - variance	Cumulative - Eigenvalue	Cumulative - %
7.8077	53.6315	7.8077	53.6315
5.2562	35.4022	13.0639	89.0337
0.9361	10.9663	14.0000	100.0000



1-pH, forested; 2-humus, forested; 3-nitrogen, forested; 4-phosphorus, forested; 5-potassium, forested; 6-pH, deforested; 7-humus deforested; 8-nitrogen, deforested; 9-phosphorus, deforested; 10-potassium, deforested; t-temperature; H-relative humidity; v-wind velocity; Pp-precipitations. The arrow represents the direction of the weighting of soil analyzed traits.

Figure 4. The representation in PC1 x PC2 plans of the variables corresponding to nutritional traits of soil located in forested and deforested areas

Potassium (5 and 10) shows a strong contribution along PC1 indicating significant variation between forested and deforested soils phosphorus (4 and 9) and nitrogen (3 and 8) also display distinct orientations emphasizing their role in differentiating soil fertility conditions pH (1 and 6) and humus (2 and 7) exhibit moderate variability suggesting that these traits are influenced by biomass removing but to a lesser extent. Environmental factors indicate their individual influence on soil nutritional dynamics. The separation of soil traits between forested and deforested areas highlights the nutrient depletion and soil fertility decline associated with biomass removing while climatic factors contribute to additional variability in soil composition.

CONCLUSIONS

The comparative analysis of soil properties in forested and deforested areas reveals the profound impact of biomass removing on soil structure and fertility. In forested areas, the soil maintains a higher clay content and total porosity, which contribute to improved water retention and aeration, while the lower apparent density ensures a more stable and less compacted structure. In contrast, deforested areas experience an increase in raw sand content and a decrease in fine particles, leading to soil degradation, compaction, and reduced infiltration capacity. The marked decline in porosity and the increase in apparent density highlight the detrimental effects of vegetation removal on soil stability.
The analysis of soil fertility further confirms the negative consequences of biomass removal. In forested soils, higher pH, greater humus content, and significantly increased nitrogen, phosphorus, and potassium levels indicate a nutrient-rich environment capable of sustaining plant growth and microbial activity. However, deforested areas show a notable reduction in these essential nutrients, with humus depletion and increased variability in phosphorus and nitrogen suggesting instability due to erosion and diminished nutrient retention. The lower pH in deforested areas points to potential acidification, reinforcing the idea that biomass removing accelerates soil degradation and reduces its ability to support vegetation.
Cluster analysis and principal component analysis (PCA) confirm that biomass removing introduces significant structural and compositional changes in the soil.
The hierarchical clustering of soil physical traits reveals strong differentiation in total porosity and clay content, while dust fractions, sand distribution, and apparent density exhibit internal similarities across environments. Nutritional trait clustering highlights potassium and phosphorus as the most affected elements, with nitrogen, humus, and pH showing smaller variations. PCA results indicate that

environmental factors, particularly biomass removing and climatic conditions, account for most of the variance in soil properties.

The findings emphasize that biomass harvesting, which is not followed by reforestation or other land use in agroforestry sector, leads to soil compaction, reduced porosity, and significant nutrient depletion, ultimately decreasing soil fertility and increasing susceptibility to erosion. The interaction between environmental and climatic factors further influences soil dynamics, reinforcing the importance of sustainable land management strategies.

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https://www.isric.org/.

Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

IMPACT OF DIGESTATE AND COMPOST APPLICATIONS ON SOIL CHEMICAL CHARACTERISTICS AND METALS AVAILABILITY IN A CONTAMINATED SOIL

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Abstract

Applying organic amendments such as digestate and compost is increasingly recognized as a sustainable strategy for improving soil quality, particularly in contaminated sites. This study investigates the effects of digestate and compost applications on the chemical characteristics of heavy metals-polluted soil and their influence on metal availability. The research was carried out in the greenhouse conditions, using maize as test plant. The main parameters analyzed were pH, organic carbon (Corg), total nitrogen (Nt), mobile phosphorus (P_{AL}), mobile potassium (K_{AL}), and the bioavailable forms of heavy metals (Pb, Cd, Cu, Mn, Ni, and Zn). Results revealed that both amendments enhanced soil pH, P_{AL}, and K_{AL}, reducing the bioavailability of heavy metals. The compost has proven to have very good performance in decreasing the mobility of heavy metals, mainly Cu, Mn, Ni, and Zn. This study highlights the suitability of these organic amendments for remediating polluted soils and improving soil fertility, offering a sustainable approach to land management in polluted areas.

Key words*: digestate, compost, soil fertility, contamination/pollution, heavy metals.*

INTRODUCTION

Soil is a vital and limited resource. The economy, the environment, and the society depend on healthy soils, which hold over 20% of the planet's biodiversity, are the largest terrestrial carbon pool, and provide water and habitats for all living organisms (Decaëns et al., 2006; FAO, 2020; EC, 2021).

Veerman et al. (2020) estimated in their report for the European Commission (EC) that approximately 60 to 70% of soils in the European Union (EU) are not healthy, a statistic also detailed in the EC's Impact Assessment Report (EC, 2008).

Healthy soils are essential for achieving climate neutrality; therefore, the EU implemented a new Soil Strategy (EC, 2021), which aims to restore degraded soils and remediate contaminated sites. If effectively implemented, this strategy could significantly improve the health of EU soils. The European Environment Agency (EEA) estimated, in 2021, that around 2.8 million land sites were contaminated in the EU (EEA, 2022). Soil contamination can occur due to human activities or natural causes and involves different contaminants, such as pesticides, ammonia and nitrate, herbicides, petroleum hydrocarbons, and heavy metals (Neethu et al., 2021; Panico et al., 2023). The most frequent contaminants are mineral oils and heavy metals (EEA, 2022).

Heavy metal contamination presents a serious threat since the presence of metals such as lead (Pb), cadmium (Cd), copper (Cu), manganese (Mn), nickel (Ni) and zinc (Zn) can inhibit plant growth and contaminate the food chain, impacting both human and animal health (Tchounwou et al., 2012; Panagos et al., 2013; Jaishankar et al., 2014; Liu et al., 2018; Panico et al., 2023).

Various *in situ* and *ex situ* remediation techniques have been developed to restore heavy metal-contaminated sites, such as surface capping, encapsulation, landfilling, soil flushing, soil washing, electrokinetic extraction,

stabilization, solidification, vitrification, phytoremediation, and bioremediation (Khalid et al., 2017; Liu et al., 2018).

The immobilization of heavy metals in soil involves various physical and chemical processes that reduce their bioavailability and mobility. The main mechanisms by which heavy metals can be immobilized include: i) *precipitation* which is based on increasing soil pH by adding alkaline amendments contributing to the precipitation of heavy metals (Park et al., 2011a; Karalić et al., 2013); ii) *complexation* – organic amendments can form complexes with heavy metals, reducing their mobility and preventing their uptake by plants (Alam et al., 2020); iii) *adsorption* – the physical adsorption of heavy metals onto soil particles can lead to their immobilization (Nartey et al., 2014; Li et al., 2021); and iv) *microbial activity* – through processes such as biosorption and bioaccumulation, certain bacteria and fungi can uptake heavy metals and immobilize them in less harmful forms (Khalid et al., 2017; Azhar et al., 2022).

One effective, well-documented and tested approach to mitigate soil contamination is the application of organic amendments (Khan et al., 2004; Khalid et al., 2017). These materials improve soil structure and fertility (Sobhi et al., 2024) and play a vital role in immobilizing heavy metals, reducing their availability to plants (Park et al., 2011a; Omokaro et al., 2024). The use of organic amendments (OAs')is increasingly seen as a viable strategy for remediating contaminated soils, aligning with the EU's Soil Strategy to restore degraded lands (EC, 2021).

As is already known, the supply of organic matter contributes to improving soil's physical and chemical properties as well as soil's biological activity (Bolan et al., 2014; Khalid et al., 2017). Additionally, organic matter (OM) acts on the mobility of heavy metals in the soil, thereby reducing their negative impact on soil microorganisms (Venegas et al., 2015; Khalid et al., 2017).

In the 1990s, one of the most polluted sites in Europe was the city of Copşa Mică, and it remains the most polluted city in Romania until present. Two factories, Carbosin, which produced carbon black, and Sometra, a non-ferrous metallurgical smelter, were behind this pollution. Carbosin shut down in 1993, but the smelter is still operational (Vrînceanu et al., 2009; Muntean et al., 2010; Stanescu et al., 2017).

The objective of this study was to evaluate the comparative effects of digestate and compost applications on the chemical characteristics of metal-contaminated soil and their influence on metal availability. The assessment of metal availability was carried out only by using the specific chemical extraction with DTPA - TEA - $CaCl_2$ (Diethylenetriaminepentaacetic acid – Triethanolamine – Calcium chloride solution). The experiment is part of a research work that aims to restore soil health by reducing heavy metal mobility, improving nutrient reserves, and ensuring favorable conditions for plant development in contaminated soils.

The effects of the OAs on important soil indicators, such as soil pH, N, C_{org}, P_{AL}, K_{AL}, and the content of heavy metals (in accessible forms to plants), were evaluated within the experiment.

MATERIALS AND METHODS

Soil and organic amendments' physiochemical characteristics

The soil used for our study was collected from a 0-20 cm depth in an Aluvisol soil type, in an area heavily impacted by heavy metal contamination. This agricultural land is located approximately 1,500 meters from the exhaust chimney of the former Sometra industrial facility in Copşa Mică. The soil samples were air-dried and milled, and the chemical characteristics and heavy metals content were analyzed (Table 1).

The OAs' and the soil used in the experiment were chemically analyzed for their nutrient content (N, P, K, and OM), contaminants (heavy metals), and total salt content (TSC). The results are also presented in Table 1.

The digestate used was produced by the Alexandru Baciu farm's biogas production unit, and the compost was obtained by co-composting sheep manure with pruning lavender residues and residues from the distillation of lavender stalks, as described by Jafri et al. (2024). The soil is characterized by a low nutrient content and a high content of Cd, Pb, and Zn. The Cd, Pb, and Zn content of the soil used in our experiment exceeds the alert thresholds for

sensitive use of land imposed by Order No. 756/1997 issued by the Ministry of Waters, Forests and Environmental Protection (3 mg/kg for Cd, 50 mg/kg for Pb, and 300 mg/kg Zn).

It should be noted that the values of C_{org}, OM, and nutrients (P and K) content established for the two types of OA are significantly different.

Table 1. Loss-of-ignition (LOI), chemical characteristics, and heavy metal contents of soil and OAs used in the experiment

Parameter	U.M.*)	Values		
		Soil	Compost	Digestate
pH		6.12	8.03	8.13
DM	%	-	56.03	26.84
LOI	%	-	46.45	51.30
OM	%	-	46.77	89.31
N_t	%	0.12	1.85	2.13
C_{org}	% dm**)	1.135	16.1	38.9
P	% dm	-	1.35	0.58
K	% dm	-	3.88	1.29
P_{AL}	mg/kg	29.5	-	-
K_{AL}	mg/kg	83.0	-	-
Cd	mg/kg	14.8	0.515	0.246
Cu	mg/kg	46.6	27.9	219
Cr	mg/kg		25.6	4.31
Ni	mg/kg	24.5	18.1	4.04
Mn	mg/kg	511	447	138
Pb	mg/kg	339	17.9	0.762
Zn	mg/kg	708	185	99.5

*) U.M. – unit of measurement
**) dm – dry matter

Soil and OAs' chemical analysis

The soil and OAs' samples underwent air-drying and grinded and were then passed through a 2 mm sieve prior to chemical analysis. The soil and OAs' physicochemical characteristics and heavy metal content were analyzed using ICPA methodologies (1981, 1983, 1986). The pH was measured potentiometrically using a pH meter in a 1:2.5 w/v soil and 0.5:2.5 w/v OAs' and deionized water extract. The analysis of dry matter (DM), loss on ignition (LOI), and organic matter (OM) was performed using the ICPA methodology (1983). Total nitrogen (N_t) was determined using a Vario MACRO Cube Elemental Analyser. P_{AL} and K_{AL} were determined in ammonium lactate acetate extraction. For P_{AL}, a colorimetric method was used, while K_{AL} was determined using flame atomic emission spectrometry (STAS 7184 19-82) based on the Egner-Riehm method (Tănase et al., 2022). C_{org} content was determined by the Walkley-Black titrimetric method, and nitrate nitrogen (NO_3^--N) was determined potentiometrically according to the ICPA methodology (1983).

The pseudo-total concentrations of metals were determined in the soil samples collected before the application of treatments using atomic absorption spectrometry after extraction by the aqua regia-microwave digestion method. Microwave digestion was performed using the Ethos Easy Microwave Digestion System, with 10 mL of aqua regia (7.5 mL HCl and 2.5 mL HNO_3) at 140°C for 30 min. The method was developed according to SR ISO 11466:1999. The same method was used for the analysis of the pseudo-total metal concentrations in the OAs' (Vrînceanu et al., 2019).

DTPA-extractable heavy metals (Pb, Cd, Cu, Mn, Ni, and Zn) were obtained by extracting 10g of soil with 20 ml of an extraction solution (0.05 M DTPA, 0.01 M $CaCl_2$, and 0.1 M tetraethylammonium, buffered to pH 7.3) following the SR ISO 14870:2002 and SR ISO 11047:1998 protocols. The extracted samples were then analyzed for available heavy metal content using a GBC Avanta 932AA flame atomic absorption spectrometer (Vrînceanu et al., 2017).

All laboratory analyses were conducted in triplicate, and the reported results represent the averages of these three replicates.

Pot experiment

A pot experiment was conducted in the greenhouse of the National Research and Development Institute for Soil Science, Agrochemistry and Environmental Protection – ICPA Bucharest, Romania. The soil was thoroughly mixed with 20 g, 40 g, and 80 g (equivalent to 15 t/ha, 30 t/ha, and 60 t/ha) of each OA, resulting in six treatments. The experiment was carried out using a randomized design with three replicates for each treatment. Additionally, two control pots were made, resulting in a total of twenty pots. Felix hybrid of maize (*Zea mays* L.) from the National Research and Development Institute for Agriculture Fundulea was used as a test plant.

The soil used for sowing was watered before planting; after that, 10 seeds were planted per pot. The plants were watered regularly to maintain the soil moisture content.

After successful germination, the plants were thinned out, with five plants remaining per pot.

At the end of the testing period, all plants were harvested, and soil samples were taken from each pot for further analysis.

Statistical analysis

Analysis of variance (ANOVA) was performed for statistical analysis, followed by Tukey`s range test (honesty significant difference). The variability in the collected data was indicated by the standard deviation ($p < 0.05$).

RESULTS AND DISCUSSIONS

The effects of OAs application on pH, N_t, N-NO_3, C_{org}, P_{AL}, K_{AL}, and TSC

Organic amendments typically influence various soil parameters, such as pH, Nt, N-NO_3, Corg, P_{AL}, K_{AL}, and TSC.

pH

Organic amendments can alter soil pH, which in turn can enhance the immobilization of heavy metals, as it is a factor governing the behavior of heavy metals in the soil-plant system (Hossain et al., 2010; Uchimiya et al., 2010; Park et al., 2011b). The pH of the soil from our experiment changed regardless of the dose or type of OAs applied. Significant increases in pH values were noted due to the increase in the quantity of OAs' regardless of its type, starting with the application of an equivalent amount of 30 t/ha. However, the application of compost, regardless of the applied dose, resulted in a significant increase in pH values compared to digestate (Figure 1). (De Vries & Breeuwsma, 1987; Kingery et al., 1994; Zaller & Köpke, 2004; van Herwijnen et al., 2006; Chang et al., 2007; Das et al., 2023).

N_t, N-NO_3, and C_{org}

The immobilization of heavy metals can be influenced by N_t, C_{org}, and N-NO_3. Their interactions facilitate microbial activities, enhance the formation of stable complexes, and contribute to the improvement of soil structure. As detailed by Liu et al. (2021), these elements facilitate metal immobilization through multiple mechanisms, including chemical stabilization, wherein heavy metals are transformed into less soluble and less toxic forms, and physical encapsulation, whereby heavy metals are sequestered within a low-permeability matrix (Liu et al., 2021).

No significant variations in soil nitrogen content were observed in our experiment, regardless of the amendment type (Figure 1). Consistent with findings from similar studies, nitrogen derived from OAs' is not readily available to plants, with only 5–15% of the N_t input from compost becoming plant-accessible (Reimer et al., 2023). The application of digestate and compost exerted comparable effects on the soil's C_{org} content as those recorded for N_t content. As illustrated in Figure 1, there were no significant differences between the compost and digestate treatments, regardless of the application rate. However, comparison to the unfertilized control, a significant increase in soil nitrogen content was recorded in the treatment corresponding to an OA application rate equivalent to 60 t/ha.

The N mineralization rate in the soil fertilized with OAs is reflected in the N-NO_3 content. In this experiment, an increase in the N-NO_3 content values was noted up to the application of quantities equivalent to the dose of 30 t/ha. After applying the equivalent amount of 60 t/ha of organic fertilizer, both in terms of compost and digestate, a decrease of N-NO_3 content was observed to values similar to those determined in the control (Figure 1).

P_{AL} and K_{AL}

Analyzing the variation in the P_{AL} and K_{AL} contents of the soil treated with the two types of

fertilizer, a sharp increase in the values of these nutrients is observed due to the increase in the amounts of compost (Figure 1). Moreover, a significant increase in P_{AL} content values is noted after applying compost (regardless of the applied dose), approximately 2.6 times higher than the value obtained after applying digestate. The significant increases in the values of the P_{AL} content compared to the control are mainly due to the P input from the compost. It is noted that when quantities of compost equivalent to 60 t/ha were applied, the increase compared to the control was over 3.7 times.

In the case of K_{AL}, applying compost resulted in significant increases compared to digestate application, regardless of the applied dose. A sharp increase in the values of the K_{AL} content, compared to the control, is observed even from the application of the first dose of compost (Figure 1).

The application of OAs' generally enhances the availability of P and K in soils. The combined effect of P_{AL} and K_{AL} can enhance the immobilization of heavy metals. Phosphorus aids in transforming metals into insoluble complexes, while potassium supports plant health and influences soil chemistry. Together, they enhance microbial activity, improve soil structure, and create a conducive environment for effective heavy metal immobilization, resulting in the formation of stable metal-organic complexes that significantly lower metal mobility (Radziemska et al., 2020). Different studies have shown that high phosphorus levels can effectively bind heavy metals to soil organic matter, reducing their availability (Ruby et al., 1994; Hettiarachchi et al., 2000; Basta et al., 2001; Cao et al., 2002; Maenpaa et al., 2002; Borlan et al., 2003; Zwonitzer et al., 2003; Amanullah Mahar et al., 2015; Seshadri et al., 2017; Andrunik et al., 2020), and mobilize heavy metals, such as Pb, Cd (Chen et al., 2024), and Zn (Andrunik et al., 2020) into less soluble forms a phenomenon also observed in our experiment (Figure 2).

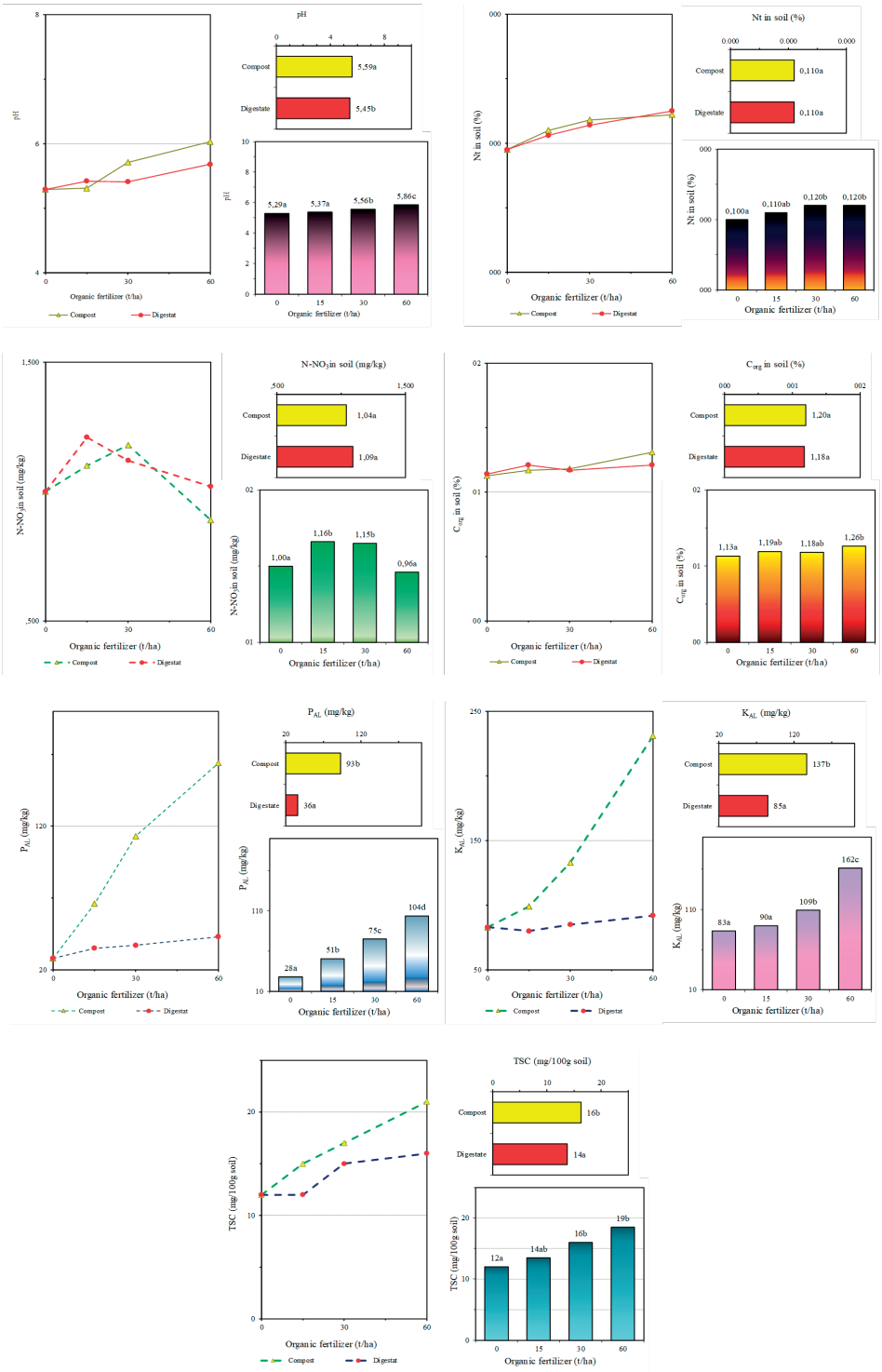
The application of P and K has been shown to influence soil pH, where the resulting increase in pH from phosphorus and potassium amendments tends to enhance the precipitation of heavy metals (Liu et al., 2021; Ou et al., 2018), in agreement with the observations of the present study.

Total salt content

Compost and digestate application can also introduce significant amounts of salts into soils, limiting plant development. Therefore, it is important to monitor this indicator in the case of long-term application of OAs, such as compost or digestate. Regarding the data obtained through this experiment, it was observed that, in the case of application of compost, TSC increased 0.8 times compared to digestate (Figure 1). Regardless of the type of OA used, increasing the applied dose led to raised values of TSC compared to the control, starting with applying a quantity equivalent to the dose of 30 t/ha.

The effects of OAs' application on heavy metals

When compost is applied over a long period of time, the issue of accumulation of potentially toxic elements such as Zn, Ni, and Cu may arise. While plant uptake of these contaminants is not frequently measured when compost is applied, their accumulation in the soil is a risk often associated with compost application (Amlinger et al., 2003; Weissengruber et al., 2018).



*Values followed by the same letter (a, b, c) do not differ significantly from each other (Tukey multiple comparison method - significance threshold α=0.05).

Figure 1. Effects of compost and digestate application on pH, N_t, N-NO_3, C_{org}, P_{AL}, $K_{AL,}$ and TSC

Cadmium

Cd is the seventh most toxic metal that can remain in the soil for several decades (Jaishankar et al., 2014). Being an extremely mobile element in the soil-plant system, the risk of translocation is high. It is essential to limit its transfer from the soil solution to crop plants by acting on the soil factors that govern the behavior of Cd in the soil. The supply of OM enhanced the retention of the metal in the soil's adsorbent complex, reducing the mobile fraction of Cd in the soil, that is, the fraction accessible to plants. Our experimental data indicate a decrease in the extractable Cd content. There are no significant differences due to the type of OAs (Figure 2). Previous studies have indicated that the available concentration of Cd in soil increases with decreasing soil pH (Li et al., 2014; Yu et al., 2016; Hou et al., 2019), which supports our findings.

Zinc

Zn is considered an essential element for plant development, which serves as a structural element for certain enzymes and is involved in numerous physiological processes (Gupta & Kalra, 2006; Ciobanu, 2019). Zn ions can have both beneficial and toxic effects on plant cells. The main factor influencing the distribution of zinc in the soil is pH, which essentially affects the mobility of Zn ions. As soil pH decreases, the solubility and absorption of Zn increase, thereby raising the potential for phytotoxicity. (Ciobanu, 2019). Compared to the control, the values of available Zn content in soil decrease significantly after applying an OA quantity equivalent to 60 t/ha.

Copper

Cu is an essential element for plant development, but its presence in the culture substrate can cause phytotoxicity. Experimental data reveals significant differences in terms of extractable Cu content in DTPA between the compost and digestate variants. The application of compost produced a significant decrease in the extractable Cu content compared to digestate, regardless of the applied dose (Figure 2). Data from the literature indicate that Cu toxicity occurs at relatively high Cu concentrations, which vary depending on the plant species (Barbosa et al., 2013; Alobaidi et al., 2015; Shun et al., 2018).

Manganese

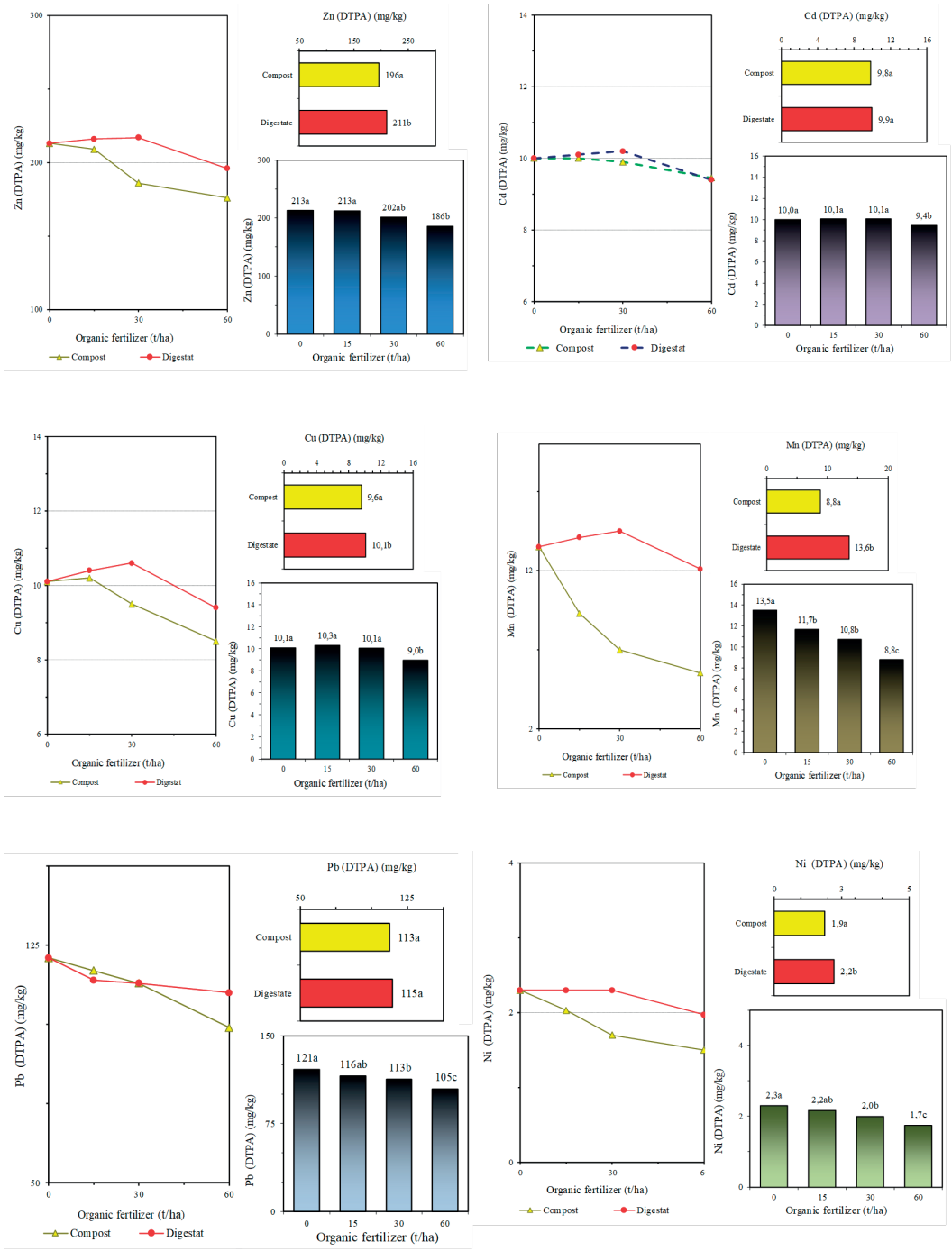
Mn is also an essential element for plant development. The soil material used in the experiment does not contain excessive Mn; however, its high solubility leads to the accumulation of high amounts of Mn in the soil solution under acidic conditions.

The compost application resulted in a significant decrease in soluble Mn content compared to the application of digestate, regardless of the applied dose. The reduced mobility of Mn in the soil solution, caused by the increased OM content from the application of organic amendments, is evidenced by a significant decrease in the DTPA-extractable Mn content as the dose of applied OAs increases (Figure 2). The lowest value was determined in the variants with the maximum dose of organic amendment (60 t/ha, regardless of the type of amendment).

Lead and Nickel

Pb and Ni are elements of no importance in plant development, unlike other metals, such as Zn, Cu, and Mn. Their presence in high quantities causes toxicity to plants, suppressing the overall growth (Jaishankar et al., 2014; Najeeb et al., 2017), inhibiting the iron uptake (Yongsheng et al., 2011; Jaishankar et al., 2014), and reducing the fertile potential of the soil. The application of organic amendment, regardless of the type, resulted in a significant reduction in mobile Ni content and mobile Pb content (Figure 2), consistent with the findings of Katoh et al. (2016). There are significant differences between the effects produced by digestate and those produced by compost, the application of compost leading to a more pronounced decrease of the mobile Ni contents.

In the case of Pb, although the application of organic amendments decreased the metal's mobility in the soil, the values of extractable Pb contents from the soil were still high.



*Values followed by the same letter (a, b, c) do not differ significantly from each other (Tukey multiple comparison method - significance threshold α=0.05).

Figure 2. Effects of compost and digestate application on accessible heavy metal (Zn, Cd, Cu, Mn, Pb, Ni) contents (extractable in DTPA - TEA - $CaCl_2$)

CONCLUSIONS

Both compost and digestate used in our study, have emerged as important amendments for remediating contaminated soils and enhancing their chemical properties. The study demonstrates that these amendments can notably improve various soil parameters, including pH, C_{org}, N_t, and the availability of key nutrients and heavy metals. The research shows that both digestate and compost can enhance soil pH and nutrient retention, thereby positively contributing to plant growth and soil health. Specifically, enhancing soil pH can increase nutrient availability, regardless of the type of organic amendment used. Despite the benefits, it is essential to consider the potential risks associated with the long-term application of compost, particularly regarding the accumulation of potentially toxic elements in the soil. The beneficial role of OAs' in immobilizing heavy metals adds complexity to soil management strategies. The ability of these amendments to improve soil fertility and reduce the bioavailability of harmful metals is to be considered in addressing soil contamination issues. Regular assessments of nutrient dynamics and heavy metal concentrations in treated soils are important for sustainable agricultural practices. Additionally, integrating these findings into broader soil conservation policies will help mitigate the adverse effects of industrial activities contributing to soil degradation.

ACKNOWLEDGEMENTS

This research work was financed from Project ADER 18.1.1 Project: Development of solutions to restore soil health in conditions of maintaining environmental sustainability by using organic fertilizers, Funder Romanian Ministry of Agriculture and Rural Development.

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***Operational STAS by ASRO – Romania.

Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

DYNAMICS OF FUNCTIONAL MICROBIOME IN POLLUTED SOIL AFTER ONE YEAR OF BIOREMEDIATION

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Abstract

Heavy metal pollution is a worldwide major concern. It represents a threat to humans, animals, plant health, and has negative impact on the environment. The Biolog Ecoplate approach was used to observe the daily changes in soil functional microbiome, in 5 sites from Baia mare exposed to historical pollution and after one year of bioremediation. The study has shown that EcoPlates are an effective tool for the assessment of microbial activity in heavy metal contaminated soils in Baia Mare. Microbial activity and diversity decreased with the degree of pollution, highlighting the impact of contamination on the soil microbiome. Within the analysed functional guilds, the values extracted showed a high range of activities. Polymers guild showed the highest activity in CT site, with a similar trend of carbohydrates and the maximum form of activities. Site FR induced the highest activity for carboxylic & acetic acids and amino acids, while the AWCD recorded its maximum.

Key words*: heavy metal, pollution impact, microbiome changes, guild activity.*

INTRODUCTION

In recent years, soil pollution by heavy metals has become a global concern due to their high toxicity, inability to biodegrade and tendency to accumulate over the long term (Fajardo et al., 2019). Heavy metals not only affect soil fertility, but also alter bacterial communities, causing a significant reduction in biodiversity (Pan et al., 2020). The diversity and number of sensitive soil bacteria may decrease, while resistant bacteria manage to adapt and multiply, leading to the formation of a specific bacterial community structure (Liu et al., 2020). Heavy metal contamination constantly influences bacterial biomass and activity (Kavamura and Esposito., 2010).

Soil is the substrate of terrestrial ecosystems and plays an important role in maintaining their balance. It has multiple functions, that include the sustain of food systems, the regulation of climate and water resources, and provide a habitat for many life forms (Ponge et al., 2015). This resource enable the continuous production of food for an increased population, maintain the biodiversity and biomass yields, along with carbon sequestration (Silver et al., 2021; Tahat et al., 2020). In Europe, there are estimated approximately 2.5 million sites with potentially contaminated soils, with heavy metals considered some of the most common contaminants (Pb, Cd, Cu, Zn) (Pérez et al., 2018). This leads to significant problem due to their lack of biodegradation, which lead to ecotoxicity and the cumulative behaviour (Fajardo et al., 2019; Pan et al., 2020).

Various research has highlighted that the presence of heavy metals in soil cause adverse effects on the soil microbiome, which lead to compromised soil functions (Xu et al., 2017; Jacquiod et al., 2018; Zhao et al., 2019). Heavy metal pollution generates stress that can influence the characteristics of bacterial communities (Lin et al., 2016).

Microbial communities in soil contribute significantly to nutrient cycling, soil fertility enhancement and carbon sequestration, which are essential for sustaining soil functionality (Fierer et al., 2017).

Toxic stress caused by heavy metals and metalloids significantly affect the diversity and structure of soil microbial communities (Zhao et al., 2019) and alters ecosystem functions (Bai et al., 2020). The metal contaminants impact functional genes and perturb soil ecological functions mediated by specialized

groups (Yin et al., 2015; Liu et al., 2018). Also, the interaction between soil microorganisms and heavy metals influences their oxidation state and lead to immobilization process that link them to organic compounds (Ahemad et al., 2019; Hou et al., 2020). This interaction has a direct impact on the bioavailability and ecotoxicity of metals (Gadd, 2024), and some bacteria can synthesize chelated compounds with siderophores that attach to metals such as Cd, Pb and Zn, leading to the formation of bio precipitates with low bioavailability (Gadd, 2024; Silva et al., 2024).

At opposite, increased species diversity in soil communities act to soil strength and resilience improvements (Shu et al., 2021).

The Biolog method is a viable used technique for analysing the metabolic activity and catabolic potential of bacterial communities (Dror et al., 2020). The Biolog Ecoplate system includes a microplate with 96 wells, each containing 31 different types of carbon substrates (Gryta et al., 2014). Community-level physiological profiling (CLPP) provides a simple and efficient method to assess the natural variation and diversity of soil microbial communities (Gomez and Garland, 2012; Gao et al., 2018). This method is based on the capacity of microorganisms to utilize various organic sources (Epelde et al., 2015; Li et al., 2015) and allows the effects of soil management practices and pollution on microbial communities to be highlighted (Gryta et al., 2014; Epelde et al., 2015; Li et al., 2015).

The study of microorganisms resistant to heavy metals and metalloids has proven useful in bioremediation research in contaminated areas (Hu et al., 2021). However, there is currently limited data on the relationship between the diversity, structure and function of soil microbial communities in relation to total or bioavailable metal species (Guo et al., 2017; Tipayno et al., 2018; Zeng et al., 2020).

The aim of this research was to assess the effect of bioremediation on the functional microbiome from historical polluted soils with heavy metals. The objectives were designed as an in-depth gradient of functional microbiome analysis. The first objective assess the microbial functional guild activity as shaped by one year of bioremediation. The second objective show the impact of bioremediation on site-specific microbial functional groups. The third objective analyse the stability and similarity of functional groups and guilds assemblages under the impact of bioremediation.

MATERIALS AND METHODS

The Biolog Ecoplate approach was used to observe the daily changes in soil functional microbiome, in 5 sites from Baia Mare exposed to historical pollution and after one year of bioremediation that are identified by an abbreviation: CR-Craica, CT-Colonia Topitorilor, FR-Ferneziu, ROMP-Romplumb and URB-Urbis.

Soil samples were collected in 2023 from an ongoing experiment initiated in 2019 across the five mentioned sites, that are located inside the town of Baia Mare (47°39′ N 23°34′ E) in North-West Romania, covering a total area of 7.3 hectares of brownfields.

The locations exhibit varying degrees of heavy metal soil contamination due to human-caused pollution, primarily stemming from mining, metallurgical activities, and urban development.

For EcoPlate examinations, soil samples and protocols adhered to the approach outlined by Weber et al., 2009, arranging all substrates according to their chemical resemblance. The solution introduced into EcoPlates underwent dilution up to 10^{-4}, and measurements were taken at 590 nm using a plate reader. The entire procedure spanned five days, reaching the plateau phase wherein no further increments were noted in the readings.

Functional groups and guilds used in the description of microbial metabolic activity follow the proposal of Stoian et al., 2022. Based on chemical similarity, an EcoPlate cand provide information on 5 different large guilds: Carbohydrates (CH); Polymers (P); Carboxylic & acetic acids (CX); Amino acids (AA) and Amines/amides (AM) to which is added the SUM, as total metabolic activity, Water – as basal activity which can be used as a control and the Average well color development (AWCD).

All data were analysed in RStudio (r Core Team, 2024), with formulas from packages “psych” (Revelle, 2024) for basic statistics.

ANOVA and LSD test were performed in "agricolae" package (de Mendiburu, 2023) to identify the differences between sites and all data were additionally explored in NMDS ordination ("vegan" package, Oksanen et al., 2022).

RESULTS AND DISCUSSIONS

Functional group analysis shows significant differences between sites, reflecting notable variations in metabolic activity. CH1 group have recorded the highest value (1.47) in the Craica site and the lowest in the Romplumb site (0.67), indicating a major contrast between the two locations. Similarly, CH2 group, had a high value (1.93) in the Craica site, while the lowest value remains in the Romplumb site (0.81). For CH3 functional microbiome the conditions in, Colonia topitorilor site stimulated the activity, while all the rest of the sites showed values of activity 3-4 times lower (Table 1).

The functional microbiome of CH4, had the highest value in the Colonia Topitorilor site and in the Romplumb site the lowest, and interesting aspect being the high activity recorded in ferneziu site. CH5 group activity show a separation between the sites, with activities that decrease gradually and only two sites that show values under 1.00. CH6, shows the highest value in the Colonia Topitorilor site and Romplumb the lowest, suggesting a possible environmental influences. The microbiome associated with CH7 presented the highest value in the Craica site (2.56), followed by Ferneziu site, both with significant higher activities compared to Romplub site. CH8 shows a different variation trend, with Ferneziu site showing the highest activity value and both Romplumb and Urbis sites with significantly lower values. For both CH9 and CH10 functional groups, the highest value was recorded in the Colonia Topitorilor site, while Romplumb site remains with a similar low activity, an observation that highlight a reduced metabolic efficiency, due to environmental conditions.

Table 1. Site-specific differences in metabolic activity of carbohydrates functional groups

	CH1	CH2	CH3	CH4	CH5	CH6	CH7	CH8	CH9	CH10
5_CR	1.47± 0.14a	1.93± 0.39a	0.51± 0.02c	0.92± 0.26bc	1.23± 0.27bc	0.74± 0.19b	2.56± 0.14a	1.53± 0.35ab	0.87± 0.33abc	0.61± 0.07b
5_CT	1.40± 0.05a	2.22± 0.21a	2.01± 0.21a	1.50± 0.07a	1.41± 0.01a	1.63± 0.06a	1.94± 0.08ab	1.93± 0.03a	1.41± 0.11a	0.98± 0.08a
5_FR	1.23± 0.05a	1.61± 0.22ab	1.11± 0.23b	1.31± 0.20ab	1.20± 0.06ab	0.95± 0.18b	2.40± 0.12a	2.08± 0.42a	1.19± 0.19ab	0.77± 0.09ab
5_ROMP	0.67± 0.12b	0.81± 0.18b	0.44± 0.09c	0.34± 0.02d	0.50± 0.01d	0.71± 0.16b	0.78± 0.17c	0.53± 0.08c	0.37± 0.03c	0.36± 0.03c
5_URB	0.80± 0.07b	1.09± 0.16b	0.68± 0.06bc	0.73± 0.12cd	0.90± 0.11cd	0.65± 0.10b	1.23± 0.59bc	0.91± 0.14bc	0.62± 0.06bc	0.62± 0.04b

Note: Means ± s.e. followed by different letters indicate significant differences between sites at $p<0.05$ LSD test. Legend: CR - Craica; CT - Colonia Topitorilor; FR - Ferneziu; URB - Urbis; ROMP - Romplumb. N-Acetyl-d-glucosamine - CH8; Pyruvic acid methyl ester - CH1; d l-α-Glycerol phosphate - CH10; d-Cellobiose - CH2; d-Xylose - CH5; β-Methyl-d-glucoside - CH4; i-Erythritol - CH6; d-Mannitol - CH7; α-d-Lactose - CH3; Glucose-1-phosphate - CH9.

Substrate analysis reveals differences between Carboxylic acids groups, with significant fluctuation in metabolic activity between te analysed sites (Table 2). The functional microbiome of CX1 and CX2 presented high activities in Ferneziu site, as an opposite to Romplumb site where were recorded the lowest ones, indicating a clear contrast between locations. Concerning CX3, Ferneziu (2.20) and Romplumb (0.70) show the largest differences, Urbis location presenting a similar low value. For CX4 group, in Ferneziu site was recorded an average activity significantly higher than the one recorded in Romplumb, scored with the lowest value (0.37). For CX5 microbiome the separation between sites is clear, with the highest value in the Craica site, while both Urbis and Romplumb showed almost two times lower activities.

The analysis of CX6 group shows a pronounced contrast between Ferneziu (2.97) and Romplumb (0.81) activities, similar to CX7, where Ferneziu reaches the highest value. For both CX8 and CX9 groups, the Colonia

Topitorilor offers good conditions for proliferation, compared to Romplumb site. An interesting aspect was observed for CX9 group, where the activity recorded in Craica and Ferneziu sites was similar to the one recorded in Colonia Topitorilor, revealing the generalist microbiome associated with this substrate. The generaltrend indicate Ferneziu as a site that sustain high functional activities, while the lowactivities form Romplumb location reflect the restrictions in metabolic potential of microbiome. The metabolic activity of soil microorganisms is influenced by the presence of heavy metals, leading to changes in their chemical behaviour, bioavailability and mobility in the environment (Selvi et al., 2019). The microbial diversity in soils is associated with distinct metabolic functions, along with bacterial ability to convert toxic metals into stable organic compounds or their chemical reduction (Wang and Chen, 2006) that sustain potential proliferation of these specialized microbial groups.

Microorganisms play an important role in biogeochemical cycles and their influence on the environment (Zhang et al., 2023; Ma et al., 2021). Under conditions of heavy metal pollution, the complexity of these biogeochemical cycles is influenced by the expression of functional genes involved in microbial carbon, nitrogen, phosphorus and sulfur processes, as well as genes that enable microorganisms to resist to heavy metals, which are essential for their metabolic adaptation (Li et al., 2021).

Regarding the results recorded in the five analysed sites, metabolic variability indicates that metabolism was an adaptive one where the highest activities were recorded, or sensitive to environmental constraints based on the lowest values. Moreover, environmental factors have a direct influence on metabolic expression, and the metabolic differences observed may represent key mechanisms by which organisms adapt to changing conditions (Prabhakaran et al., 2016).

Table 2. Site-specific differences in metabolic activity of carboxylic acids functional groups

	CX1	CX2	CX3	CX4	CX5	CX6	CX7	CX8	CX9
5_CR	1.90±	0.79±	1.84±	0.69±	2.07±	2.53±	1.39±	0.72±	1.49±
	0.12a	0.17b	0.16a	0.03bc	0.07a	0.11ab	0.61b	0.04b	0.36a
5_CT	1.83±	1.53±	1.59±	1.71±	1.91±	2.26±	2.36±	1.08±	1.63±
	0.07a	0.04a	0.16a	0.06a	0.19a	0.19b	0.06a	0.06a	0.22a
5_FR	1.89±	1.73±	2.20±	0.92±	1.75±	2.97±	3.06±	0.78±	1.70±
	0.09a	0.24a	0.20a	0.25b	0.34a	0.02a	0.15a	0.10b	0.04a
5_ROMP	0.41±	0.37±	0.70±	0.37±	0.85±	0.81±	0.37±	0.32±	0.37±
	0.04c	0.03b	0.30b	0.03c	0.40b	0.40c	0.03c	0.04c	0.01b
5_URB	0.97±	0.59±	0.72±	0.69±	0.73±	0.86±	0.61±	0.62±	0.94±
	0.15b	0.06b	0.04b	0.07bc	0.10b	0.14c	0.08bc	0.12b	0.42ab

Note: Means ± s.e. followed by different letters indicate significant differences between sites at $p<0.05$ LSD test. Legend: CR - Craica; CT - Colonia Topitorilor; FR - Ferneziu; URB - Urbis; ROMP - Romplumb. α-Keto butyric acid - CX8; d-Galactonic 2-Hydroxy benzoic acid - CX4; acid γ-lactone - CX2; γ-Hydroxy butyric acid - CX6;d-Galacturonic acid - CX3;; 4-Hydroxy benzoic acid - CX5; d-Glucosaminic acid - CX1; Itaconic acid - CX7;; d-Malic acid - CX9.

The analysis of Polymers and Amino acids groups shows significant differences between the activities recorded (Table 3). Colonia Topitorilor and Ferneziu frequently show the high values, while Romplumb and Urbis show the lowest ones. P1, P2, P3 and P4 groups, have the highest values in the Colonia Topitorilor site while Romplumb has the lowest values, indicating significant differences.

The AA1 functional microbiome reaches the highest value of 2.37 in the Ferneziu site, while in Urbis decrease significantly to 0.66. A similar pattern also occurs for AA2 and AA4, where the highest values are in Ferneziu site, followed by Craica (AA2) and Colonia Topitorilor (AA4). For AA3 microbiome, the most suitable conditions were recorded in Colonia Topitorilor, in contrast with the lowest values recorded in Romplumb site. For the functional microbiome of AA5 and AA6, Colonia Topitorilor and Romplumb remains in opposite places, emphasizing the consistent differences between these sites and the ability of microbiome to proliferate.

In the amine category, AM1, Ferneziu has the highest average value, while Urbis has the lowest average one (Table 4). Therefore, there was observed a significant difference between

these two sites in term of specialized microbiomes. As for the AM2 group, the maximum value recorded is 1.75 (Colonia Topitorilor), while the lowest value of 0.64 is identified for Romplumb. The assessment of the basal community (WAT) revealed the highest functional activity in Colonia Topitorilor, while Romplumb site presented a significant lower one. The other three sites recorded significant intermediate activities compared to the extremes.

Table 3. Site-specific differences in metabolic activity of polymers and amino acids functional groups

	P1	P2	P3	P4	AA1	AA2	AA3	AA4	AA5	AA6
5_CR	1.67±	1.40±	0.58±	0.77±	2.33±	2.74±	1.23±	1.36±	0.70±	0.66±
	0.02a	0.25ab	0.01bc	0.17cd	0.48a	0.25a	0.24bc	0.35bc	0.14bc	0.07c
5_CT	1.85±	1.81±	1.7±	1.73±	1.84±	2.02±	2.21±	2.11±	1.51±	1.48±
	0.09a	0.05a	0.14a	0.07a	0.46a	0.33a	0.11a	0.16ab	0.23a	0.20a
5_FR	1.63±	1.44±	1.50±	1.56±	2.37±	2.77±	1.57±	2.15±	0.93±	1.04±
	0.06a	0.08a	0.06a	0.19ab	0.15a	0.07a	0.41ab	0.31a	0.06b	0.08b
5_ROMP	0.72±	0.90±	0.38±	0.49±	0.75±	0.88±	0.49±	0.76±	0.36±	0.36±
	0.27b	0.16b	0.03c	0.12d	0.21b	0.41b	0.10c	0.22c	0.01c	0.01c
5_URB	0.81±	0.90±	0.81±	1.11±	0.66±	0.67±	0.88±	0.67±	0.59±	0.66±
	0.03b	0.16b	0.13b	0.13bc	0.07b	0.09b	0.28bc	0.09c	0.04bc	0.06c

Note: Means ± s.e. followed by different letters indicate significant differences between sites at $p<0.05$ LSD test. Legend: CR - Craica; CT - Colonia Topitorilor; FR - Ferneziu; URB - Urbis; ROMP - Romplumb.; Glycogen - P4; Tween 80 - P2; Glycyl-l-glutamic acid - AA6;α-Cyclodextrin - P3; l-Arginine - AA1; l-Phenylalanine - AA3; l-Serine - AA4; l-Asparagine - AA2; l-Threonine - AA5; Tween 40 - P1.

Table 4. Site-specific differences in metabolic activity of basal activity and amines functional groups

	AM1	AM2	WAT	AWCD
5_CR	0.72±	1.08±	0.58±	0.74±
	0.04b	0.38ab	0.04b	0.02b
5_CT	1.90±	1.75±	0.80±	0.94±
	0.12a	0.02a	0.07a	0.10ab
5_FR	2.11±	1.29±	0.56±	1.09±
	0.09a	0.14ab	0.02b	0.01a
5_ROMP	0.75±	0.64±	0.33±	0.23±
	0.36b	0.22b	0.01c	0.09c
5_URB	0.65±	0.67±	0.59±	0.18±
	0.05b	0.08b	0.07b	0.04c

Note: Means ± s.e. followed by different letters indicate significant differences between sites at $p<0.05$ LSD test. Legend: CR - Craica; CT - Colonia Topitorilor; FR - Ferneziu; URB - Urbis; ROMP - Romplumb. Putrescine - AM2; WAT – basal respiration;; Phenylethylamine - AM1; AWCD - Average Well Color Development.

High levels of CH7 in Craica soils indicate increased microbial activity, associated with an efficient decomposition of organic substrates (Figure 1). In contrast, low CH4 values in Romplumb soils may reflect an inhibition of microbial activity, associated with heavy metal toxicity. A high activity of polymers decomposition, especially P1 in Ferneziu soils, suggests a significant number of microorganisms capable of degrading complex compounds. On the other hand, low values of P3 in Craica soils suggest a reduced microbial diversity and a limited capacity to degrade polymeric substances, most likely due to heavy metal contamination. High levels of carboxylic and acetic acids, such as CX7 in Ferneziu soils, indicate the activity of microbial communities under stress conditions. However, low values of CX8 in Romplumb soils could be a sign of microbial inhibition caused by heavy metal toxicity. High amounts of amino acids such as AA4 in Ferneziu soils show active protein synthesis and favourable metabolic condition of microorganisms. In contrast, the lower AA6 values in Romplumb soils suggest a potential metabolic stress due to metal contaminants, which interfere with protein biosynthesis. High levels of amines and amides, such as AM1 in Ferneziu soils, indicate an increased microbial activity related to protein degradation, while low AM2 values in Urbis soils may suggest an inhibition of microbial functions due to the presence of heavy metals.

Regarding the functional guilds, as associated similar microbiomes, it was observed that the highest value is recorded for the CX guild (Table 5). In contrast, the lowest value can be associated with the amine category guild or AM. This contrast between the two guilds reflects the significant differences in specific parameters (Table 5).

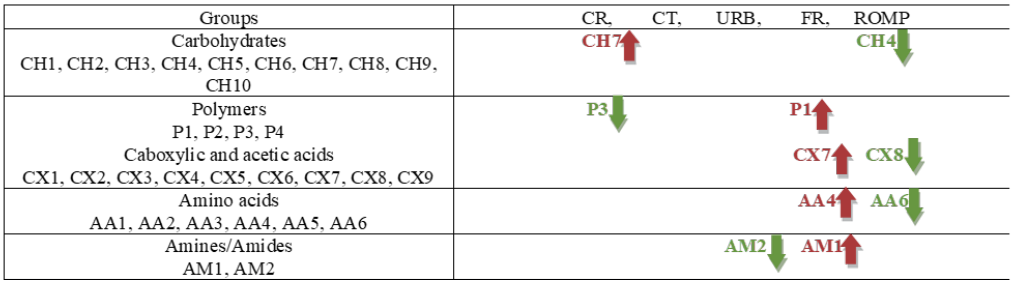


Figure 1. Variation trends in microbial functional groups as affected by historical pollution with heavy metals.
Legend: CR - Craica; CT - Colonia Topitorilor; FR - Ferneziu; URB - Urbis; ROMP - Romplumb

Table 5. Site-specific differences in metabolic activity of community and functional guilds

	SUM	P	CH	CX	AA	AM
5_CR	41.1±	4.42±	12.43±	13.46±	9.04±	1.80±
	2.20b	0.30b	1.99b	0.80b	0.25a	0.37b
5_CT	54.3±	7.1±	16.47±	15.9±	11.1±	3.65±
	1.00a	0.32a	0.30a	0.77ab	0.64a	0.14a
5_FR	51.3±	6.15±	13.90±	17.0±	10.8±	3.4±
	0.60a	0.08a	0.69ab	0.33a	0.79a	0.22a
5_ROMP	17.7±	2.49±	5.57±	4.62±	3.62±	1.41±
	2.96c	0.51c	0.69c	1.04c	0.91b	0.59b
5_URB	24.1±	3.64±	8.28±	6.77±	4.16±	1.32±
	3.31c	0.32b	1.31c	0.94c	0.64b	0.13b

Note: Means ± s.e. followed by different letters indicate significant differences between sites at $p<0.05$ LSD test.Legend: CR - Craica; CT - Colonia Topitorilor; FR - Ferneziu; URB - Urbis; ROMP - Romplumb. Polymers (P); SUM - sum of all activity recorded; Amino acids (AA) and Amines/amides (AM); Carboxylic & acetic acids (CX); Carbohydrates (CH);

The NMDS ordination project the variations in microbial activities and community similarities in each of the different heavy metal polluted sites in Baia Mare. The analysis was performed based on the site locations, the distances within site-specific communities and between different sites, along with the guild vectors, which indicates the metabolic pathways of the microorganisms (Figure 2). The central point of the NMDS projection highlights the main carbon sources available, such as amino acids, amines, polymers, carbohydrates, carboxylic acids, which are situated along the axis 2. The Craica site is located in the center of the ordination, indicating a moderate microbial activity in soils with a relative low pollution. It is positive correlated with vectors AM and AA, suggesting high metabolic activity and a diverse microbial community capable of using a wide range of substrates. A higher concentration of amines and amino acids is observed, while CX and CH are present with lower activities. The proximity to the P vector indicates that the microbial diversity shows an affinity towards polymers.

Colonia Topitorilor is located close to the Ferneziu site, which suggests a similar microbial composition in these two locations. It is related to vectors CX and P, but is not directly related to AA, indicating low utilization levels for these last substrates. This association reflects a moderate level of heavy metal pollution. The microbial community at this site appears less diverse in comparison to Craica, with an ability to adapt to pollution. In case of the lower heavy metal pollution, this allows the microorganisms to avoid metabolizing amino acids.

Ferneziu shows similar microbial activity, but with some differences. It has a strong link with AM, which means that the microorganisms here utilize them at higher rates. However, it is

also associated with CX, which suggests that these substrates are strongly decomposed. This shows that the microorganisms in Ferneziu site are adapted to decompose both AM and CX substrate groups, suggesting that they have been exposed to pollution for a long time
Romplumb is significantly different from the other sites, because is the most isolated on the ordination. This position shows that it has a unique microbial community affected by heavy heavy metal pollution. Because it has no specific links to the substrates (AA, CX, P, CH, AM), it is interpreted that toxic pollution has decreased microbial diversity and forced them to adapt to these difficult conditions. This difference suggests that the microorganisms in Romplumb are either highly specialized or that their activity is limited by toxic stress. The community is composed of heavy metal-resistant species and showed lower values of metabolic activities, which indicates the difficulties encountered by microorganisms to persist in this environment.

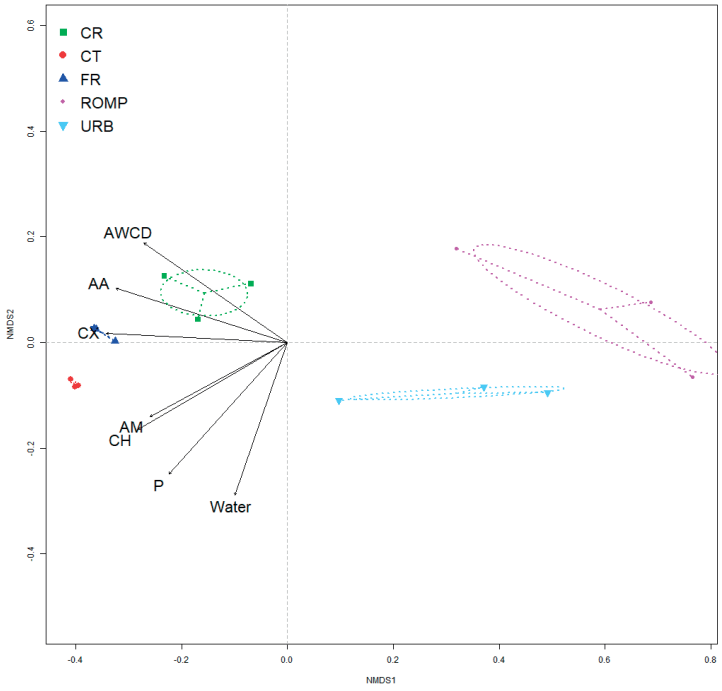


Figure 2. Variation trends in microbial functional groups as affected by historical pollution with heavy metals. Legend: CR - Craica; CT - Colonia Topitorilor; FR - Ferneziu; URB - Urbis; ROMP - Romplumb; AA - Amino acids; AM -Amines/Amides; P - Polymers; CH - Carbohydrates; CX - Carboxylic & acetic acids; SUM - total metabolic activity; AWCD - Average well color development; WATER - basal activity

Urbis is also different from the other sites, but it is closer to the center of the ordination than Romplumb, indicating that pollution has a moderate impact. Regarding the fact that this site is not related to the analysed vectors, its location indicates a combined influence of heavy metal pollutants. Microorganisms at this site are affected by pollution stress, which reduces their diversity and activity. Heavy metal pollution supports the growth of specialized microorganisms that can tolerate contaminants, but their metabolic activity is more affected than in sites with lower heavy metal pollution.

CONCLUSIONS

Microbial activity and diversity decreased with the degree of pollution, showing the impact of contamination on the entire soil microbiome.

Polymers guild showed the highest activity in Colonia Topitorilor site, with a similar trend of carbohydrates and the maximum form of activities.

Site Ferneziu induced the highest activity for CX and AM, while the AWCD recorded its maximum.

The most intense polymer and carbohydrate activity was observed in soils less contaminated with heavy metals.

There are significant differences between the microbial communities of polluted sites in Baia Mare. Craica, Colonia Topitorilor and Ferneziu sites show diverse metabolic activity, with microorganisms adapted to utilize different carbon and nitrogen sources. In contrast, Romplumb and Urbis are isolated and reflect specialized microbial communities adapted to extreme pollution conditions.

There is a visible ability of microorganisms to adapt to chemical stress in different contaminated environments, leading to site-specific functional microbiome patterns.

ACKNOWLEDGEMENTS

This paper is part of a PhD study in the thematic area of "*The traceability of heavy metals in historically polluted sites and the possibilities for their return to agriculture*", conducted by the first author B.P., under the coordination of Prof. dr. Roxana Vidican (R.V.).

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

RESEARCH ON THE INFLUENCE OF ORGANIC AND MINERAL FERTILIZATION ON THE PHYSICAL PROPERTIES OF THE SOIL IN COVASNA COUNTY

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Abstract

As an important pawn in intensive agriculture, fertilizers are identified, whether organic or mineral, whit their specific effects on the physical properties of the soil, which is what this study aims to do. The application of mineral fertilizers has a quick and predictable effect, but with higher acquisition and application costs. At the same time, for a long term, they have side effects, such as decreased soil fertility and soil toxicity. Organic fertilizers improve soil structure, texture and water retention capacity, leading to more optimal development conditions for the plant root. Also, organic fertilizers are a much more affordable option for farmers, but somehow more difficult to handle and manage. Therefore, the work will track the value of some important soil physical properties such as soil reaction, humus content, bulk density, degree of settling and porosity, in relation to different doses of organic, mineral and organo-mineral fertilizer applied in an experimental sugar beet field in Covasna County.

Key words*: compaction degree, density, mineral fertilizer, organic fertilizer, porosity, soil fertility.*

INTRODUCTION

Soil can be defined as the surface layer of the Earth's crust, consisting of mineral particles, organic matter, water, air, and living organisms. It is a highly dynamic system that performs many functions and is vital to human activities and the survival of ecosystems (Brady et al., 2008).

The action of various natural or anthropogenic factors leads to soil degradation, significantly reducing in the medium and long term the area of land that can be used for agricultural purposes (Lal, 2001). These processes of soil degradation, especially those used in agriculture, represent a serious environmental and socio-economic problem (Oldeman et al., 1990; Rounsevell et al., 2010). Thus, agricultural land represents a strategic resource, the loss of land due to various causes can generate food crises and social turmoil (Alexandratos & Bruinsma, 2012). The restrictions imposed by these limiting factors can act singly or in conjunction, resulting in a decrease in soil quality and even the cancellation of their functions. The main restrictions on the quality of agricultural soils are: drought, periodic excess of moisture, water erosion of the soil, landslides, wind erosion, excessive skeleton on the soil surface, salinization, secondary compaction due to improper work, primary soil compaction, crust formation, low-extremely low humus reserve, strong and moderate acidity, poor and very poor provision of mobile phosphorus and potassium, low content of nitrogen, deficiencies of microelements, physical and chemical pollution; covering of land with waste and solid residues.

In Covasna County, a series of limiting factors on the production capacity of soils are manifested, such as nitrogen and phosphorus deficit, which affect 111,338 ha and 102,660 ha respectively, to which are added significant areas affected by pseudo-gleyization processes (63,495 ha) and gleyization (43,564 ha), as well as potassium (49,282 ha) and organic matter (30,159 ha) deficits. Regarding the coverage of soil with waste and solid residues, it is found that 840 ha are affected by various excavation works.

Soil fertility is essential for agricultural productivity, and the use of organic and mineral fertilizers significantly influences its physical properties. Parameters such as bulk density, total porosity, and compaction degree

reflect the structural state of the soil and its ability to support crops. Studies show that adequate fertilization can improve these properties, contributing to sustainable agriculture.

The predominant soils in Covasna County are chernozems, reddish-brown soils, and podzolic soils, each with distinct physical characteristics. These are influenced by climatic factors, vegetation types, and intensive agricultural activity (Rusu et al., 2013).

On the highest peaks, brown podzolic mountain soils are found, which are characterized by high acidity and a high content of organic matter. Another type of soil is brown and brown acid forest soils, which are more widespread in the Baraolt Mountains, but also appear in islands in the Bodoc, Vrancei and Întorsurii Mountains. The largest area of Covasna County is occupied by brown and clay-iluvial podzolic soils. These soils are found especially on the wide and low peaks, as well as on the slightly inclined slopes of the Întorsurii, Vrancei, Nemira, Bodoc and Baraolt Mountains. Leached chernozems or pratazioms are found especially around the city of Târgu Secuiesc, Câmpu Frumos. In the lowest part of the county, we find hydro-morphic soils with the subgroups of gleyic, humic-gleyic soils and eutrophic peats, these being used especially for pastures and hayfields because they present a low degree of fertility and an excess of humidity, especially during the rainy periods of the year. Another category of soils that is found near the locality of Reci, on an area of approximately 18 km^2, is repre-sented by unsoilied sands, mentioned in the specialized literature as “The Dunes of Reci”.

Organic fertilization involves using compost, manure, and other organic materials that improve soil structure and increase organic matter content (Muntean et al., 2011) and mineral fertilization involves the application of chemical fertilizers providing essential elements such as nitrogen, phosphorus, and potassium. It directly impacts plant growth but can negatively affect soil structure when overused (Dobrovolski, 2001).

Effects on soil physical properties

Organic fertilization stabilizes soil aggregates and increases porosity, promoting water infiltration and reducing erosion risk. In contrast, excessive mineral fertilization can lead to soil compaction and reduced structural stability (Lal, 2004).

Organic matter improves water retention capacity by creating a porous and stable environment. Mineral fertilization, especially excessive nitrogen application, can reduce this capacity by destroying natural soil aggregates (Brady et al., 2008).

Adding compost and manure reduces bulk density and increases total porosity, facilitating root development. Chemical fertilizers can lead to soil compaction when misused (Hillel, 1998).

Organic fertilization positively affects the physical properties of the soil, improving structure, porosity, and water retention capacity. Mineral fertilization, although essential for agricultural productivity, can have negative effects on soil structure if misused. In Covasna County, the combined application of both types of fertilization represents an optimal solution for maintaining soil health.

Soil tests are extremely important before establishing a crop, as they help farmers determine whether the soil type is suitable for the plants to be grown, but also for establishing an appropriate fertilization plan.

For sampling, a spade, probe, cylinders or other available tools can be used. It is important to remember that the tools used must be clean and rust-free, so as not to contaminate the soil samples.

Regarding the soil sampling points, they can be taken in a zig-zag, diagonal or other ways, depending on the configuration of the land, being considered partial samples. There are made from 15 to 20 such partial samples.

This study analyzes how different types of fertilization influence the physical properties of the soil in Covasna County, based on collected and analyzed samples.

MATERIALS AND METHODS

Soil samples were collected at depths of 0-20 cm, from Covasna County, Zăbala village. There is also where the research takes place, on a demonstration plot of 1.5 hectares.

The climatic conditions of the area can be characterised by an average annual temperature of 10.64°C, and 500-600 mm/year precipi-tations.

The soil on which the experiment was located showed a pH value of 6.13, being classified as acidic soil. Low values were identified for potassium (K) – 13.5 mg/kg, phosphorus (P) – 10.4 mg/kg, calcium (Ca) – 36.8 mg/kg, magnesium (Mg) – 10.4 mg/kg, and zinc (Zn) – 0.6 mg/kg; normal values for sodium (Na) – 8.2 mg/kg, manganese (Mn) – 17.7 mg/kg, and boron (B) – 0.3 mg/kg; and high values were identified for iron (Fe) – 105.9 mg/kg and copper (Cu) – 1.6 mg/kg.

To assess the efficiency of mineral and organic fertilizers, the variants are compared with the control (unfertilized).

The research involves three sugar beet hybrids: Darvas, Deseda and Tatry, that were fertilized with organic, mineral and organo-mineral fertilizers as follows: 20 t/ha manure, 20 t/ha manure + 150 kg/ha N + 150 kg/ha P + 150 kg/ha K, 10 t/ha manure, 10 t/ha manure + 150 kg/ha N + 150 kg/ha P + 150 kg/ha K, only mineral fertilization with 150 kg/ha N + 150 kg/ha P + 150 kg/ha K and the control (unfertilized) (Table 1).

The mineral fertilizer used was Azomures NPK Complex 15-15-15 which is a solid fertilizer specially formulated to provide complete and balanced nutrition to plants. It contains equal proportions of nitrogen (N), phosphorus (P) and potassium (K), making it ideal for a wide range of agricultural and horticultural crops. It contributes to the vigorous and healthy growth of plants.

Table 1. The experimental factors

Fertilizer/ Hybrid	a1: DARVAS	a2: DESEDA	a3: TATRY
b1: 20 t manure/ ha	a1b1	a2b1	a3b1
b2: 20 t manure/ ha + 150 kg/ha N + 150 kg/ha P + 150 kg/ha K	a1b2	a2b2	a3b2
b3: 10 t manure/ ha + 150 kg/ha N + 150 kg/ha P + 150 kg/ha K	a1b3	a2b3	a3b3
b4: 10 t manure/ ha	a1b4	a2b4	a3b4
b5: 150 kg/ha N + 150 kg/ha P + 150 kg/ha K	a1b5	a2b5	a3b5
b6: control (unfertilized)	a1b6	a2b6	a3b6

The physical properties analyzed were: bulk density (Da, g/cm³) – indicates the degree of compaction and soil porosity, total porosity (PT, %) – reflects the soil's ability to retain water and air and compaction degree (GT, %) – provides information on structural changes in the soil relative to a reference state.

The soil samples were collected using metal cylinders from each variant and repetition of the study, in November 2024.



Figure 1. Metal cylinders with a volume of 100 cm^3

The cylinders were marked and numbered to distinguish each other, and the samples collected were later analyzed at the soil science laboratory of the University of Agronomic Sciences and Veterinary Medicine of Bucharest (Figures 1 and 2).



Figure 2. Collected soil samples in metal cylinders

RESULTS AND DISCUSSIONS

Bulk density (g/cm³)

Soil bulk density is the ratio of the mass to the bulk or macroscopic volume of soil particles plus pore spaces in a sample (Blake, 1965).

Bulk density of a soil is a dynamic property that varies with the soil structural conditions. In general, it increases with profile depth, due to

changes in organic matter content, porosity and compaction (Chaudhari et al., 2013).

At the average level, the lowest bulk density value was recorded for variant a2b1 - 1.17 g/cm³, respectively the Deseda hybrid fertilized with 20 t of manure/ha. The control variant a2b6, unfertilized Deseda hybrid, has a bulk density of 1.33 g/cm³, the difference between a2b1 and a2b6 being -0.160 g/cm³. Following the statistical analysis, the difference between a2b1 and a2b6 is classified as very significant.

The differences between variants a1b1, a1b2, a1b3, a1b4, a2b2, a2b5, a3b1, a3b2 and their control variants are also classified as very significant.

The remaining variants, according to Table 2, are classified as having no significant differences at all in bulk density.

Table 2. Statistical analysis of bulk density data

Fact A / Fact B	The influence of experimental factors on bulk density								
	a1 - DARVAS			a2 - DESEDA			a3 - TATRY		
	Value g/cm³	Difference g/cm³	Significance	Value g/cm³	Difference g/cm³	Significance	Value g/cm³	Difference g/cm³	Significance
b1	1.267	-0.065	***	1.17	-0.160	***	1.212	-0.117	***
b2	1.207	-0.125	***	1.247	-0.082	***	1.147	-0.182	***
b3	1.315	-0.017	***	1.382	0.052	-	1.342	0.012	-
b4	1.305	-0.027	***	1.362	0.032	-	1.330	0.001	-
b5	1.375	0.042	-	1.277	-0.052	***	1.335	0.005	-
b6	1.333	Mt	-	1.33	Mt	-	1.33	Mt	-
Dl 5%= 0.113; Dl 1%= 0.151 Dl 0.1%= 0.197									

Total porosity (%)

The geometry of the pore system of soil is just as complex as that of the solid phase.

Porosity is the percentage of the soil volume occupied by pore spaces. The types of pores present in a soil are as important as the amount of pore space. Pore spaces are filled with air or water. Pore space is divided into different categories by pore diameter, especially the large soil pores that are associated with the transfer and movement of both water and air. In many cases, pore-size distribution is considered the best indicator of the soil physical condition (Hao et al., 2008).

Organic fertilization contributes to increasing soil porosity, favoring microbiological activity.

Regarding total porosity, the largest very significant difference was identified at variant a1b5, the Darvas hybrid fertilized with 150 kg/ha N + 150 kg/ha P + 150 kg/ha K, respectively 45.03%, 2.29% less than the unfertilized control variant of this hybrid, a1b6, which recorded 47.32% total porosity.

Very significant differences were also recorded at variants a1b4, a2b3, a3b4, a3b3, a3b5 compared to the specific control of each variant.

Distinctly significant differences were marked at variants a2b1 and a3b2 with variations ranging between 6.37% and 7.14% compared to the control variants of each hybrid.

According to Table 3, only the value obtained by the Tatry hybrid fertilized with 20 t of manure/ha marked significant differences, due to the 4.61% difference between its value, 51.45%, and the value of the unfertilized control variant, i.e. 46.85%.

Table 3. Statistical analysis of total porosity data

fact A / fact B	The influence of experimental factors on total porosity (%)								
	a1 - DARVAS			a2 - DESEDA			a3 - TATRY		
	Value %	Difference %	Significance	Value %	Difference %	Significance	Value %	Difference %	Significance
b1	49.39	2.07	-	53.13	6.37	**	51.45	4.61	*
b2	51.71	4.38	-	50.11	3.64	-	53.99	7.14	**
b3	47.34	0.02	-	44.63	-2.13	***	46.23	-0.61	***
b4	47.22	-0.09	***	45.58	-1.18	***	46.96	0.11	-
b5	45.03	-2.29	***	49.01	2.24	-	46.55	-0.29	***
b6	47.32	Mt	-	46.76	Mt	-	46.85	Mt	-
Dl 5%= 4.572 Dl 1%= 6.10 Dl 0,1%=7.991									

The compaction degree (%)

Soil compaction affects hydraulic properties, and thus can lead to soil degradation and other adverse effects on environmental quality (Zhang et al., 2006).

Soil compaction is an important component of the land degradation syndrome which is an issue for soil management throughout the world.

The principal causes are when compressive forces derived from wheels, tillage machinery and from the trampling of animals, act on compressible soil. Compact soils can also be found under natural conditions without human or animal involvement. Compaction alters many soil properties and adverse effects are mostly linked to a reduction in permeability to air, water and roots (Batey, 2009).

The application of organic fertilizers can reduce soil compaction, improving its permeability.

Regarding the soil compaction degree, Tatry hybrid fertilized with 20 t of manure / ha + 150 kg/ha N + 150 kg/ha P + 150 kg/ha K (a3b2) recorded the percentage value of -14.985%, thus marking the greatest difference compared to the unfertilized Tatry control (a3b6), respectively -15.217%, which marks a very significant difference.

Very significant differences are recorded for each of the three hybrids studied, namely: Darvas a1b1, a1b2, a1b3 and a1b4; Deseda a2b1, a2b2 and a2b5; Tatry a3b1 and a3b4.

The remaining variants, according to Table 4, are classified as having no significant differences at all in compaction degree.

Table 4. Statistical analysis of compaction degree data

Fact A / Fact B	The influence of experimental factors on compaction degree (%)								
	a1 - DARVAS			a2 - DESEDA			a3 - TATRY		
	Value %	Difference %	Significance	Value %	Difference %	Significance	Value %	Difference %	Significance
b1	-5.192	-5.097	***	-13.162	-13.572	***	-9.585	-9.817	***
b2	-10.132	-10.037	***	-6.717	-7.127	***	-14.985	-15.215	***
b3	-8.32	-0.737	***	4.955	4.545	-	1.542	1.31	-
b4	-5.85	-0.487	***	4.427	4.017	-	-0.015	-0.247	***
b5	4.09	4.185	-	-4.375	-4.785	***	0.86	0.627	-
b6	-0.095	Mt	-	0.41	Mt	-	0.232	Mt	-
Dl 5%=9.691 Dl 1%=12.942 Dl 0.1%=16.939									

CONCLUSIONS

Organic fertilization positively affects the physical properties of the soil, improving structure, porosity, and water retention capacity. Mineral fertilization, although essential for agricultural productivity, can have negative effects on soil structure if misused. In Covasna County, the combined application of both types of fertilization represents an optimal solution for maintaining soil health.

The study highlighted that fertilization influences the physical characteristics of the soil, especially bulk density and total porosity.

The results suggested that:

- the analyzed soil has a structure favorable for cultivation, but there are differences between fertilization variants;
- optimal bulk density and adequate porosity can be maintained by using organic fertilizers, which improve soil structure and support microbiological activity;
- continuous monitoring of the soil's physical properties is essential for maintaining long-term fertility.

Following the measurements, Deseda (a2b3) and Tatry (a3b2) variants registered the highest and the lowest values of the elements analyzed.

The fact that Deseda a2b3 had the highest average value of bulk density (1.38 g/cm³) was subsequently reflected in the total porosity index, which recorded the lowest average value (44.63%). The highest value of the compaction degree for Deseda a2b3 (4.95%) variant occurred as an effect of the deficient values of the other targeted elements.

Tatry a3b2 has the lowest average values in terms of bulk density (1.15 g/cm³), which is why total porosity managed to reach such a high average value (53.99%). Regarding the compaction degree for Tatry a3b2 variant, the fact that it is negative (-14.98%) highlights the extremely good aeration of the soil.

ACKNOWLEDGEMENTS

This research work was carried out with the support of the University of Agronomic Sciences and Veterinary Medicine Bucharest.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

IDENTIFICATION AND CHARACTERIZATION OF SOIL RESOURCES SPECIFIC TO THE FLOODPLAIN AREA OF ROSEȚI COMMUNE, CĂLĂRAȘI COUNTY

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Abstract

The primary objective of this research is to evaluate the possibilities of maintaining the optimal natural potential of soils within a sustainable land use framework. This involves assessing the land's capacity to support various agricultural activities without compromising natural resources while ensuring long-term soil fertility. In the studied area, the floodplain of Roseți Commune, Călărași County, four main soil types were identified: Alluvisols, Gleysols, Psamosols and Anthrosols. These soils reflect ecological diversity and pedological conditions specific to floodplain areas, exhibiting a slightly alkaline pH (7.2-7.6). Alluvisols are fertile soils formed through alluvial deposits and have a moderate humus content (3.92%). In contrast, Gleysols are characterized by poor drainage and intense gleying processes due to high moisture excess, with a low humus content (1.68%). Psamosols, with a high sand content, have a reduced water retention capacity and a moderate humus supply (2.56%), whereas Anthrosols are soils modified by human activity. The identified soils are well supplied with phosphorus (65-71 ppm) and potassium (160-360 ppm), ensuring the agricultural and ecological potential of the region.

Key words*: soil, evaluation marks, quality class.*

INTRODUCTION

Land evaluation is a crucial aspect of natural resource management, playing a fundamental role in the efficient and sustainable use of agricultural, forestry, and urban land. In a context marked by climate change and increasingly diverse economic demands, the importance of this activity has grown significantly.

According to the Food and Agriculture Organization of the United Nations (FAO, 1976), land evaluation is defined as "a process by which the performance of a given land area is assessed in relation to specific uses, through the analysis of environmental, social, and economic factors." This definition was later refined by experts such as Sys et al. (1991), who emphasized that land evaluation involves "a detailed analysis of soil, climate, and topographical characteristics to determine the most suitable use of the land."

The land evaluation process considers not only physical aspects but also socio-economic elements. According to Bouma (2002), "decisions regarding land use must incorporate both the natural characteristics of the soil and the economic and social factors that influence its viability." In Romania, this process is known as "bonitation" and is used to determine the productive potential of agricultural land. Based on the methodology developed by ICPA Bucharest (1987), land is classified according to pedological, agrochemical, and climatic characteristics to establish its suitability for various crops.

Another key concept in land evaluation is the suitability of land for specific agricultural crops. As Dudal (1976) states, "land is considered suitable for a crop if it meets the crop's requirements under normal climatic conditions and with the application of standard technologies". This approach aligns with that of Klingebiel and Montgomery (1961), who defined suitability as "the extent to which land can support specific uses without requiring excessive technological interventions."

Thus, land evaluation is not merely a technical analysis of soil but a complex and interdisciplinary process, essential for the

sustainable development of agriculture and effective territorial planning.

MATERIALS AND METHODS

The soil study was conducted in two distinct phases: the field phase and the office phase, each involving specific activities for the analysis and evaluation of soil properties.

Field Phase

This stage involved activities carried out directly in the analyzed area, aimed at collecting data and samples necessary for soil evaluation. The main operations performed included:

- General examination of the land and its natural context;
- Investigation of soil profiles, correlating data with pedogenetic factors and existing agricultural conditions;
- Detailed description of soil morphological characteristics;
- Sampling for laboratory analysis.

To determine the main soil properties, the following analytical methods were applied:

- pH determination using the potentiometric method, with a soil-to-water ratio of 1:2.5;
- Mobile phosphorus analysis using the Egner-Riehm-Domingo method, based on ammonium acetate-lactate extraction;
- Mobile potassium determination using the same technique as for phosphorus;
- Humus content evaluation through the modified Walkley-Black method (Gogoasă modification);
- Base saturation degree calculation (V, %) by determining hydrolytic acidity and the sum of exchangeable base cations;
- Granulometric analysis using the Kacinsky method to establish soil fraction proportions.

Office Phase

In this stage, the data collected in the field were processed and interpreted to obtain a clear understanding of soil characteristics. The main activities conducted were:

- Delimiting soil territorial units (STUs) and classifying them according to the Romanian Soil Taxonomy System (SRTS, 2012) at both higher (type, subtype) and lower levels (variety, granulometric species, family, variant);
- Analyzing and describing soil territorial units based on field observations and laboratory results, including physico-chemical and morphological properties;
- Evaluating agricultural land using the methodology of the Research Institute for Soil Science and Agrochemistry (ICPA), with the aim of assigning bonitation scores and classifying land according to its suitability for various agricultural crops;
- Establishing land quality classes in accordance with specific agricultural uses.

Through this methodological approach, a detailed characterization of the soil was obtained, essential for sustainable land use and the optimization of agricultural practices.

RESULTS AND DISCUSSIONS

The floodplain area of Roseți Commune is located within the Danube Floodplain and is characterized by low-lying relief, dominated by marshy lands, fluvial ridges, and depressions. The predominant soils are Alluvisols, which can be weakly developed, incompletely developed, or well-developed but relatively young, formed through the deposition of alluvial sediments transported by the Danube. These soils are fertile and favorable for agriculture; however, they were historically subject to frequent flooding.

Climate and Aeolian Regime

The region has a temperate-continental climate, with hot, dry summers and cold winters. The average annual temperatures range between 10-11°C, while in July, the Danube's water temperatures can reach 25-28°C. During winter, the probability of ice formation is 83%, and ice bridges can lead to rising water levels and flooding.

The aeolian regime is influenced by local characteristics such as micro-relief and soil texture. The alluvial sands on ridges and flat surfaces warm up more rapidly in summer, leading to increased evaporation and a soil moisture deficit between June and October.

Precipitation

The average annual precipitation ranges between 450 and 550 mm, but its distribution is uneven. The moisture deficit is more

pronounced in sandy soils, where water infiltrates rapidly, whereas medium-textured soils retain moisture more effectively.

Hydrology

The hydrological regime of the area is determined by the Danube and its branches, Borcea and Rău. Hydrological studies indicate that the average slope gradient of the Danube and Borcea is 45 mm/km, while the Rău Branch has a steeper gradient.

The water flow velocity varies as follows:

- Borcea Branch: minimum 0.37 m/s, average 0.80 m/s, maximum 1.03 m/s;
- Danube: minimum 0.88 m/s, average 1.05 m/s, maximum 1.30 m/s;
- Rău Branch: minimum 1.19 m/s, average 1.28 m/s, maximum 1.36 m/s.
- The water discharge (in cm^3/s) follows this order:
- Borcea Branch: minimum 200, average 700, maximum 3,250;
- Rău Branch: minimum 1,700, average 3,100, maximum 6,750;
- Danube: minimum 2,650, average 6,150, maximum 15,800.

The average amplitude of the Danube's water level reaches 5-6 m but can exceed 8.8 m during floods. The annual sediment transport is estimated at approximately 70-80 million tons.

Following hydrotechnical works for embankment and drainage, flooding has been controlled, and excess water from precipitation and river overflow is discharged through an open-channel drainage network. In addition to drainage, irrigation is essential for maintaining agricultural productivity by ensuring a controlled water supply tailored to soil characteristics and existing crops.

Vegetation and Land Use

Embankment and drainage have significantly transformed the floodplain, allowing the expansion of agricultural land. Natural vegetation has largely been replaced by cultivated crops; however, wetland areas still support specific species such as common reed (*Phragmites australis*), cattail (*Typha* spp.), sedge (*Carex* spp.), as well as trees like willows (*Salix* spp.) and poplars (*Populus* spp.).

Currently, maize (*Zea mays*) is the dominant crop due to the soil's natural fertility, irrigation, and the beneficial influence of the groundwater table. Additionally, wheat (*Triticum aestivum*), sunflower (*Helianthus annuus*), sugar beet (*Beta vulgaris*), and soybean (*Glycine max*) are cultivated, taking advantage of the floodplain's favorable conditions.

Profile 1 - Mollic Gleyic Alluviosols
Latitude - N: 44°09'44"
Longitude - E: 27°25'54"
Major relief unit: Romanian Plain
Unit: Bărăganului Plain
Parental material: carbonate loessoid deposits
Groundwater: 2-3 m
Current use: Arable
Representative profile: 27

Morphological characterization (Figure 1)

Apk (0-18 cm): sandy clay, grayish-brown (10 YR 5/2) when moist, light grayish-brown (10 YR 6/2) when dry, slightly moist, structure disturbed by agricultural activities, friable, weakly cohesive, slightly plastic, weakly adhesive, rare small macropores, frequent thin herbaceous roots, strong effervescence, clear and sharp transition.

Am (18-32 cm): clayey loam, brown (10 YR 5/3) when moist, pale brown (10 YR 6/3) when dry, slightly moist, small granular structure, friable, weakly cohesive, slightly plastic, weakly adhesive, weakly compact, rare small macropores, frequent thin herbaceous roots, strong effervescence, gradual transition.

Go (32-68 cm): clayey loam, yellowish-brown (10 YR 3/4) when moist, yellowish-brown (10 YR 5/4) when dry, massive, dry, weakly compact, weakly cohesive, slightly plastic, rare thin herbaceous roots, strong effervescence, gradual transition.

Gr (68-118 cm): sandy clay loam, yellowish-brown (10 YR 5/4) with olive spots (2.5 Y 4/4) when moist, light yellowish-brown (10 YR 6/4) with light olive-brown spots (2.5 Y 5/4) when dry, massive, weakly compact, weakly cohesive, slightly plastic, strong effervescence throughout, gradual transition.

CkGr (118-150 cm): fine sandy loam, brownish-yellow (10 YR 6/6) with light olive-brown spots (2.5 Y 5/4) when moist, brownish-yellow (10 YR 6/8) with light olive spots (2.5 Y 5/6) when dry, slightly moist, massive, weakly compact, weakly cohesive, slightly plastic, strong effervescence throughout.

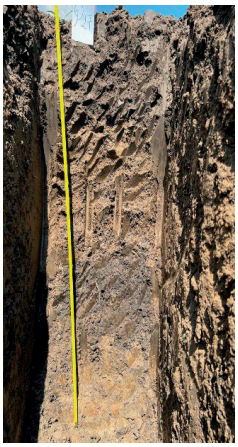


Figure 1. Mollic Gleyic Alluviosols

The soil reaction is slightly alkaline, with pH increasing from 7.2 at the surface to 7.7 at deeper layers. The humus supply is moderate, ranging from 3.92% to 2.16% in the upper layers of the profile. The mobile phosphorus content at 0-18 cm is 71 ppm, indicating a well-supplied soil, while mobile potassium is 160 ppm, also demonstrating sufficient potassium levels (Table 1).

The soil profile consists of varying textures, including sandy clay, clayey loam, and sandy loam. The shallow groundwater table (2-3 m) has a slight impact on evaluation scores. The humus reserve is high, at 210 t/ha, which positively influences the soil's evaluation.

Evaluation scores for several crops are as follows:

Wheat, barley, sunflower, peas, and beans: 81 points, placing them in the 1st quality class and the 2nd favorability class.

Corn: 80 points, placing it in the 2nd quality class and the 3rd favorability class.

Soybean: 73 points, placing it in the 2nd quality class and the 3rd favorability class (Table 2).

The potato crop scores the lowest, with 45 points, placing it in the 3rd quality class and the 6th favorability class. Sugar beet scores 57 points, placing it in the 3rd quality class and the 5th favorability class.

The average evaluation for all eight crops is 72 points, classifying the land in the 2nd quality class and the 3rd favorability class.

Table 1. The maine physical and chemical properties

Horizon	Depth (cm)	Physical properties				Texture class	Chemical properties				
		Coarse sand (2-0.2 mm)	Fine sand (0.2-0.02 mm)	Dust (0.02-0.002 mm)	Colloidal clay (<0.002 mm)		pH (H_2O)	$CaCO_3$ (%)	Humus (%)	P_{AL} (ppm)	K_{AL} (ppm)
Ap	0-18	0.43	48.23	13.04	38.30	TN	7.2	-	3.92	71	160
Am	18-32	0.34	46.42	18.94	34.30	TT	7.3	9	3.88	-	-
Go	32-68	0.57	42.73	22.00	34.70	SF	7.4	14	2.16	-	-
Gr	68-118	0.59	60.24	19.57	19.60	SF	7.6	9.8	-	-	-
CGr	118-150	1.17	62.58	17.85	18.40	SF	7.7	16.4	-	-	-

Table 2. Land Suitability for the main crops

Crop	Tem 3C	Pre 4C	Gl 14	Stg 15	Sal/Alc 16/17	Text 23	Pol 29	Slo 33	Ls 38	HL 39	Flo 40	TP 44	$CaCO_3$ 61	pH 63	EV 133	HR 144	EM 181	EM
Wheat	1	0.9	1	1	1	1	1	1	1	0.9	1	1	1	1	1	1	1	81
Barley	1	0.9	1	1	1	1	1	1	1	0.9	1	1	1	1	1	1	1	81
Maize	1	0.8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	80
Sunflower	1	0.9	1	1	1	1	1	1	1	0.9	1	1	1	1	1	1	1	81
Potato	0.8	0.7	1	1	1	0.9	1	1	1	0.9	1	1	1	1	1	1	1	45
Sugar beet	0.9	0.7	1	1	1	0.9	1	1	1	1	1	1	1	1	1	1	1	57
Soybean	0.9	0.9	1	1	1	1	1	1	1	0.9	1	1	1	1	1	1	1	73
Peas/Beans	1	0.9	1	1	1	1	1	1	1	0.9	1	1	1	1	1	1	1	81

Note: average annual temperature - 3C, average annual precipitation - 4C, gleization - 14, stagnogleization - 15, salinization or alkalization - 16/17, texture - 23, pollution - 29, slope - 33, landslides - 38, hydrostatic level - 39, floodability - 40, total porosity - 44, total $CaCO_3$ content - 61, soil pH-63, edaphic volume - 133, humus reserve - 144, surface soil moisture excess - 181.

Profile 2 - Calcaric Gleysols
Latitude - N: 44°09'25"
Longitude - E: 27°29'17"
Major relief unit: Romanian Plain
Unit: Bărăganului Plain

Parental material: carbonate loessoid deposits
Groundwater: 1-2 m
Current use: Arable
Representative profile: 43



Figure 2. Calcaric Gleysols

Morphological characterization (Figure 2)
Apk (0-20 cm): clay loam, grayish-brown (10 YR 5/2) when moist, light grayish-brown (10 YR 6/2) when dry, structure partially disturbed by agricultural activities, moist, friable, slightly plastic, slightly adhesive, weakly compact, few medium macropores, thin herbaceous roots, moderate effervescence, clear and sharp transition.
Aok Go (20-46 cm): clay loam, brown (10 YR 4/3) with olive-brown mottles (2.5 Y 4/4) when moist, pale brown (10 YR 6/3) with light olive-brown mottles (2.5 Y 5/4) when dry, moist, structureless, friable, moderately plastic, moderately adhesive, compact, frequent thin herbaceous roots, frequent medium macropores, strong effervescence, gradual transition.
Grk1 (46-58 cm): clay loam, dark yellowish-brown (10 YR 5/4) with frequent olive mottles (5 Y 4/3) when moist, yellowish-brown (10 YR 5/4) with frequent olive mottles (5 Y 4/4) when dry, polyhedral structure, moist, moderately plastic, moderately adhesive, weakly compact, rare thin herbaceous roots, strong effervescence, gradual transition.
Gr2 (58-125 cm): clay loam, yellowish-brown (10 YR 5/6) with frequent olive mottles (5 Y 5/3) when moist, pale yellowish-brown (10 YR 6/4) with frequent pale olive mottles (5 Y 6/3) when dry, massive structure, moist, hard, moderately plastic, moderately cohesive, moderately compact, rare thin herbaceous roots, few medium macropores, strong effervescence throughout the matrix, gradual transition.
CkGr (125-150 cm): clay loam, yellowish-brown (10 YR 5/8) with frequent olive mottles (5 Y 5/6) when moist, brownish-yellow (10 YR 6/8) with frequent pale olive mottles (5 Y 6/4) when dry, massive structure, wet, hard, moderately plastic, moderately adhesive, weakly compact, few medium macropores, strong effervescence throughout the matrix.
Soil Properties and Evaluation. The pH increases along the soil profile, from 7.2 at the surface to 7.6 at deeper layers, indicating a slightly alkaline reaction. The humus content is low, ranging from 1.92% to 0.52% in the first 95 cm of the profile (Table 3). Mobile phosphorus at a depth of 0-20 cm is 66 ppm, showing the soil is well-supplied with this nutrient, while mobile potassium is 180 ppm, also indicating sufficient potassium availability.
Soil Suitability for Agriculture. The soil profile consists entirely of clay loam. The shallow groundwater table (1-2 m) negatively affects the evaluation marks for this area. The humus reserve is low at 92 t/ha, which also impacts the soil's agricultural potential.
Evaluation scores for various crops are as follows:
Wheat and barley: 43 points, placing them in the 3rd quality class and 6th favorability class.
Corn: 45 points, also in the 3rd quality class and 6th favorability class.
Soybeans: 39 points, placing them in the 4th quality class and 7th favorability class.
Potatoes: 21 points, placing them in the 4th quality class and 8th favorability class.
Sugar beet: 28 points, also in the 4th quality class and 8th favorability class.
Sunflower, peas, and beans: 50 points, placing them in the 3rd quality class and 6th favorability class (Table 4).
The overall evaluation for all eight crops is 40 points, classifying the land in the 4th quality class and 7th favorability class.

Table 3. The maine physical and chemical properties

Horizon	Depth (cm)	Physical properties				Texture class	Chemical properties				
		Coarse sand (2-0.2 mm)	Fine sand (0.2-0.02 mm)	Dust (0.02-0.002 mm)	Colloidal clay (<0.002 mm)		pH (H_2O)	$CaCO_3$ (%)	Humus (%)	P_{AL} (ppm)	K_{AL} (ppm)
Ap	0-20	0.54	44.26	17.00	38.20	TT	7.2	-	1.92	66	180
Ao	20-46	0.45	46.05	16.20	37.30	TT	7.2	-	1.68	-	-
Gr1	46-95	0.22	34.00	30.58	35.20	TT	7.2	-	0.52	-	-
Gr2	95-121	0.13	38.55	26.62	34.70	TT	7.4	7.0	-	-	-
CGr	121-150	17.48	72.82	9.70	10.60	TT	7.6	16.2	-	-	-

Table 4. Land Suitability for the main crops

Crop	Tem 3C	Pre 4C	Gl 14	Stg 15	Sal/Alc 16/17	Text 23	Pol 29	Slo 33	Ls 38	HL 39	Flo 40	TP 44	$CaCO_3$ 61	pH 63	EV 133	HR 144	EM 181	EM
Wheat	1	0.9	1	1	1	1	1	1	1	0.6	1	1	1	1	0.8	1	1	43
Barley	1	0.9	1	1	1	1	1	1	1	0.6	1	1	1	1	0.8	1	1	43
Maize	1	0.8	1	1	1	1	1	1	1	0.7	1	1	1	1	0.8	1	1	45
Sunflower	1	0.9	1	1	1	1	1	1	1	0.7	1	1	1	1	0.8	1	1	50
Potato	0.8	0.,7	1	1	1	0.9	1	1	1	0.6	1	1	1	1	0.7	1	1	21
Sugar beet	0.9	0.7	1	1	1	0.9	1	1	1	0.7	1	1	1	1	0.7	1	1	28
Soybean	0.9	0.9	1	1	1	1	1	1	1	0.6	1	1	1	1	0.8	1	1	39
Peas/Beans	1	0.9	1	1	1	1	1	1	1	0.7	1	1	1	1	0.8	1	1	50

Note: average annual temperature - 3C, average annual precipitation - 4C, gleization - 14, stagnogleization - 15, salinization or alkalization - 16/17, texture - 23, pollution - 29, slope - 33, landslides - 38, hydrostatic level - 39, floodability - 40, total porosity - 44, total $CaCO_3$ content - 61, soil pH - 63, edaphic volume - 133, humus reserve - 144, surface soil moisture excess - 181.

Profile 3 - Anthrosols
Latitude - N: 44°09'52"
Longitude - E: 27°28'41"
Major relief unit: Romanian Plain
Unit: Bărăganului Plain
Parental material: carbonate loessoid deposits
Groundwater: 1-2 m
Current use: Arable
Representative profile: 42
Morphological characterization (Figure 3)



Figure 3. Anthrosols

Ap (0-15 cm): Clay loam, dark grayish-brown (10 YR 5/2) when moist, light grayish-brown (10 YR 6/2) when dry; composed of transported material, slightly plastic, slightly adhesive, weakly compacted; few medium-sized macropores; numerous fine herbaceous roots; moderate effervescence; distinct and abrupt boundary.
Ao1 (15-28 cm): Clay loam, brown (10 YR 4/3) when moist, pale brown (10 YR 6/3) when dry; moist, polyhedral structure, friable, moderately plastic, moderately adhesive, compacted; numerous fine herbaceous roots; frequent medium-sized macropores; transitional boundary.
Ao2 (28-54 cm): Clay loam, dark yellowish-brown (10 YR 5/4) when moist, yellowish-brown (10 YR 5/4) when dry; moist, polyhedral structure, moderately plastic, moderately adhesive, weakly compacted; few fine herbaceous roots; slight effervescence; gradual boundary.
Go (54-117 cm): Fine sandy clay loam, yellowish-brown (10 YR 5/6) when moist, pale yellowish-brown (10 YR 6/4) when dry; massive structure, moist, hard, moderately plastic, moderately cohesive, moderately compacted; sparse fine herbaceous roots; infrequent medium-sized macropores;

moderate effervescence; gradual boundary. CGo (117-150 cm): Fine sandy clay loam, yellowish-brown (10 YR 5/8) with prominent olive mottles (5 Y 5/6) when moist, yellowish-brown (10 YR 6/8) with noticeable pale olive mottles (5 Y 6/4) when dry; massive structure, wet, hard, moderately plastic, moderately adhesive, weakly compacted; few medium-sized macropores; strong effervescence throughout.

The pH increases with depth, ranging from 7.2 to 7.6, indicating a slightly alkaline reaction. The humus content is relatively low, varying from 1.80% to 0.62% in the upper 54 cm of the soil profile. The available phosphorus concentration (0-15 cm depth) is 69 ppm, categorizing the soil as well-supplied, while the available potassium level (180 ppm) also classifies it as adequately supplied (Table 5). The soil texture changes throughout the profile, transitioning from clay loam and silty clay loam to fine sand. The presence of a shallow groundwater table (1-2 meters) negatively impacts soil assessment scores. The humus reserve is limited (103 t/ha), contributing to a reduction in evaluation marks. Crop suitability assessments indicate that wheat and barley received a score of 43 points, placing them in the third quality category and sixth favorability class. Maize also falls within the third quality category, scoring 45 points, and the sixth favorability class. Soybean is ranked in the fourth quality category with 39 points and the seventh favorability class. Potato cultivation received 24 points, placing it in the fourth quality class and eighth favorability class, whereas sugar beet scored 31 points, situating it in the fourth quality category and seventh favorability class (Table 6). Sunflower, peas, and beans achieved a score of 50 points, classifying them in the third quality category and the sixth favorability class. The overall evaluation score across the eight crops is 41 points, positioning the land in the third quality category and the sixth favorability class.

Table 5. The maine physical and chemical properties

Horizon	Depth (cm)	Physical properties				Texture class	Chemical features				
		Coarse sand (2-0.2 mm)	Fine sand (0.2-0.02 mm)	Dust (0.02-0.002 mm)	Colloidal clay (<0.002 mm)		pH (H_2O)	$CaCO_3$ (%)	Humus (%)	P_{AL} (ppm)	K_{AL} (ppm)
Ap	0-15	1.16	52.34	20.20	26.30	LL	7.2	-	1.80	69	180
Ao1	15-28	1.80	50.78	19.82	27.60	LL	7.1	-	1.29	-	-
Ao2	28-54	0.22	49.11	16.17	34.50	TT	7.3	7.6	0.62	-	-
Go	54-117	0.90	58.93	21.67	18.50	NF	7.6	21	-	-	-
CGo	117-150	1.25	57.96	23.39	17.40	NF	7.6	25.0	-	-	-

Table 6. Land suitability for the main crops

Crop	Tem 3C	Pre 4C	Gl 14	Stg 15	Sal/Alc 16/17	Text 23	Pol 29	Slo 33	Ls 38	HL 39	Flo 40	TP 44	$CaCO_3$ 61	pH 63	EV 133	HR 144	EM 181	EM
Wheat	1	0.9	1	1	1	1	1	1	1	0.6	1	1	1	1	1	0.8	1	43
Barley	1	0.9	1	1	1	1	1	1	1	0.6	1	1	1	1	1	0.8	1	43
Maize	1	0.8	1	1	1	1	1	1	1	0.7	1	1	1	1	1	0.8	1	45
Sunflower	1	0.9	1	1	1	1	1	1	1	0.7	1	1	1	1	1	0.8	1	50
Potato	0.8	0.7	1	1	1	1	1	1	1	0.6	1	1	1	1	1	0.7	1	24
Sugar beet	0.9	0.7	1	1	1	1	1	1	1	0.7	1	1	1	1	1	0.7	1	31
Soybean	0.9	0.9	1	1	1	1	1	1	1	0.6	1	1	1	1	1	0.8	1	39
Peas/Beans	1	0.9	1	1	1	1	1	1	1	0.7	1	1	1	1	1	0.8	1	50

Note: average annual temperature - 3C, average annual precipitation - 4C, gleization - 14, stagnogleization - 15, salinization or alkalization - 16/17, texture - 23, pollution - 29, slope - 33, landslides - 38, hydrostatic level - 39, floodability - 40, total porosity - 44, total $CaCO_3$ content - 61, soil pH - 63, edaphic volume - 133, humus reserve - 144, surface soil moisture. excess – 181.

Profile 4 - Psamosols
Latitude - N: 44°12'26"
Longitude - E: 27°28'34"
Major relief unit: Romanian Plain
Unit: Bărăganului Plain

Parental material: carbonate loessoid deposits
Groundwater: 2-3 m
Current use: Arable
Representative profile: 36



Figure 4. Psamosols

Morphological characterization (Figure 4)
Aț (0-7 cm): loamy sand, dark grayish brown (10 YR 3/2) when moist, light grayish brown (10 YR 3/2.5) when dry, transported material, slightly plastic, slightly adhesive, weakly compacted, with a few medium-sized macropores, thin herbaceous roots, moderate effervescence, clear and abrupt transition.
Ao (7-36 cm): coarse sand, brown (10 YR 3/3) when moist, pale brown (10 YR 3/3) when dry, compact, with 10% oxidation spots, polyhedral structure, friable, moderately plastic, moderately adhesive, compact, frequent thin herbaceous roots, frequent medium-sized macropores, moderate effervescence, gradual transition.
Go (36-63 cm): coarse sand, dark yellowish brown (10 YR 5/4) when moist, yellowish brown (10 YR 5/4) when dry, polyhedral structure, moist, slightly plastic, slightly adhesive, weakly compacted, rare thin herbaceous roots, weak effervescence, gradual transition.
Gr (63-113 cm): fine sandy clay, yellowish brown (10 YR 5/6) when moist, pale yellowish brown (10 YR 6/4) when dry, massive, moist, hard, moderately plastic, moderately cohesive, moderately compact, moderate effervescence, gradual transition.
CGo (113-150 cm): sandy clay, yellowish brown (10 YR 5/8) with frequent olive spots (5 Y 5/6) when moist, brownish yellow (10 YR 6/8) with frequent pale olive spots (5 Y 6/4) when dry, massive, wet, hard, moderately plastic, moderately adhesive, weakly compacted, few medium-sized macropores, strong effervescence.
The soil reaction is slightly alkaline, with pH ranging from 7.3 to 7.7 as the depth increases. Humus content is low, with values between 2.56% and 1.48% in the first 63 cm of the profile.
Phosphorus availability at 0-7 cm depth is 65 ppm, indicating well-supplied soil, while potassium availability is 360 ppm, classifying the soil as very well-supplied in potassium (Table 7).
The texture varies across the profile, transitioning from loamy sand to coarse sand and sandy clay, which negatively affects evaluation marks for different crops. The shallow groundwater table (2-3 m depth) also contributes to lower evaluation scores. The humus reserve is low, with a value of 120 t/ha, further impacting the evaluation results.
For wheat, peas, and beans, the evaluation score is 32 points, placing them in the 4th quality class and 7th favorability class.
Corn and sunflower are ranked in the 3rd quality class with 45 evaluation points, classified in the 6th favorability class. Soybeans are in the 4th quality class with 29 evaluation points and placed in the 8th favorability class.
Potatoes scored 28 evaluation points, placing them in the 4th quality class and 8th favorability class. Sugar beet received 31 evaluation points, placing it in the 4th quality class and 7th favorability class.
Barley scored 39 points, placing it in the 4th quality class and 6th favorability class (Table 8).
The overall evaluation score for the eight crops is 35 points, classifying the land in the 4th quality class and 7th favorability class.

Table 7. The maine physical and chemical properties

Horizon	Depth (cm)	Physical properties				Texture class	Chemical features				
		Coarse sand (2-0.2 mm)	Fine sand (0.2-0.02 mm)	Dust (0.02-0.002 mm)	Colloidal clay (<0.002 mm)		pH (H_2O)	$CaCO_3$ (%)	Humus (%)	P_{AL} (ppm)	K_{AL} (ppm)
Aţ	0-7	16.32	64.87	8.61	10.20	UM	7.3	8.8	2.56	65	360
Ao	7-36	9.96	78.63	6.51	4.90	UG	7.7	11.4	1.69	-	-
Go	36-63	11.96	76.57	6.67	4.80	UG	7.7	11.4	1.48	-	-
Gr	63-113	7.93	44.29	13.58	34.20	TN	7.6	13.0	-	-	-
CGr	113-150	2.15	48.61	13.62	35.10	TN	7.7	17.4	-	-	-

Table 8. Land suitability for the main crops

Crop	Tem 3C	Pre 4C	Gl 14	Stg 15	Sal/Alc 16/17	Text 23	Pol 29	Slo 33	Ls 38	HL 39	Flo 40	TP 44	$CaCO_3$ 61	pH 63	EV 133	HR 144	EM 181	EM
Wheat	1	0.9	1	1	1	0.5	1	1	1	0.9	1	1	1	1	1	0.8	1	32
Barley	1	0.9	1	1	1	0.6	1	1	1	0.9	1	1	1	1	1	0.8	1	39
Maize	1	0.8	1	1	1	0.7	1	1	1	1	1	1	1	1	1	0.8	1	45
Sunflower	1	0.9	1	1	1	0.7	1	1	1	0.9	1	1	1	1	1	0.8	1	45
Potato	0.8	0.7	1	1	1	0.8	1	1	1	0.9	1	1	1	1	1	0.7	1	28
Sugar beet	0.9	0.7	1	1	1	0.7	1	1	1	1	1	1	1	1	1	0.7	1	31
Soybean	0.9	0.9	1	1	1	0.5	1	1	1	0.9	1	1	1	1	1	0.8	1	29
Peas/Beans	1	0.9	1	1	1	0.5	1	1	1	0.9	1	1	1	1	1	0.8	1	32

Note: average annual temperature - 3C, average annual precipitation - 4C, gleization - 14, stagnogleization - 15, salinization or alkalization - 16/17, texture - 23, pollution - 29, slope - 33, landslides - 38, hydrostatic level - 39, floodability - 40, total porosity - 44, total $CaCO_3$ content - 61, soil pH - 63, edaphic volume - 133, humus reserve - 144, surface soil moisture excess - 181.

CONCLUSIONS

The Roseți commune, situated in the Danube Floodplain, features a low-relief terrain with fertile alluvial soils, though historically prone to frequent flooding. Soil analysis of the area revealed four distinct soil units: Mollic Gleyic Alluviosols, Calcaric Gleysols, Anthrosols, and Psamosols, each exhibiting unique ecopedological properties that impact their suitability for agricultural use.

Of these four soil units, Mollic Gleyic Alluviosols stands out for its agricultural potential, scoring an average of 72 points, which places it in the 2nd quality class and 3rd favorability class. In contrast, Calcaric Gleysols, Anthrosols, and Psamosols scored lower, with evaluation marks between 35 and 41 points, due to factors such as lower humus content, less favorable soil texture, and a shallow groundwater table.

Alluvial soils in Roseți commune are the most fertile and agriculturally suitable, particularly those classified under Soil Unit 1 (Mollic Gleyic Alluviosols), which demonstrated the highest evaluation marks for major crops and a high humus reserve of 210 t/ha. Conversely, Calcaric Gleysols, Anthrosols, and Psamosols face ecopedological challenges such as lower humus levels (1.92%, 1.80%, and 2.56%, respectively), less ideal texture, and a shallow groundwater table (1-3 m), which makes them less productive.

Crops such as wheat, barley, sunflower, peas, and beans are highly suited to this region, achieving strong evaluation marks across alluvial soils. Potatoes and sugar beet, however, are less suitable and receive low evaluation marks across all soil units due to less favorable ecopedological conditions.

The region holds substantial agricultural potential, but effective soil management practices are necessary to improve soil quality, particularly by boosting humus content and managing moisture levels. To address the identified soil limitations, several improvement strategies are recommended, including:

- Organic fertilization to raise humus levels.
- Drainage systems to manage the groundwater table.
- Strategic crop selection and land use planning to maximize productivity.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

DECREASE IN THE ACTIVITY OF SOIL FUNCTIONAL MICROBIOMES IN HEAVY METAL POLLUTED SOILS

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Abstract

The presence of heavy metals in urban soil is a consequence of anthropogenic activities and represents a continuous source of changes in microbial communities. The DEMSA model was used to analyse a series of decreases in the soil microbial functional profile, with a target on the groups and guilds that suffer a reduction due to the presence of heavy metals. The presence of heavy metals in the 5 sites analysed is visible in the reduction of the soil microbiome general activity. The Index of Narrowing is set in a very large interval, which indicates the absence of multiple functional groups from the altered community. The diversity indices show a large variability associated with the reduction of specialized functional activity. The sum of activities is a good indicator of functional contraction, with most of the functional guilds showing activities below 1.0 units, and a large variability between functional groups. Soil microbiomes show powerful changes due to the presence of heavy metals in soil, which is visible in the decrease of multiple groups functional activity.

Key words*: functional narrowing, index of contraction, indicator guild, microbial communities.*

INTRODUCTION

Historical soil pollution with heavy metals relates to the multitude of anthropogenic activities during the development of urban areas (Mehmood et al., 2020; Sharma et al., 2015). Green areas in cities possess multiple services necessary for human life, both from a physical and physiological point of view, and thus the presence of heavy metals in soil is important in the risk assessment procedures associate with human well-being (Luo et al., 2024; Poggio and Vrščaj, 2009).

For polluted soils within cities bioremediation approaches are effective methods for heavy metal removal, considering their beneficial environmental effects (Sánchez-Castro et al., 2023). An important aspect related to bioremediation effect is the continuous monitoring process, which enable the detection of the key factors that sustain its success (Liu et al., 2022; Vega et al., 2022; Zhang and Wang, 2020; Xiang et al., 2020). Phytoremediation as a bioremediation technique implies the use of plants that can remove heavy metals from soil and thus decrease their degree of pollution (Kumar et al., 2022; Tonelli et al., 2022). This process relies on microbial activity, especially in the rhizosphere of plants, which can increase the remediation effect (Yan et al., 2020).

An in-depth analysis of soil microorganism reveal that microbial components are severely affected by the presence of heavy metals (Adewumi and Ogundele, 2024; Tang et al., 2024). One of the most harmful effects is the reduction of populations and the variability of microbial activities (Horváth et al., 2021; Sager, 2020). In this context, the monitoring of microbial dynamics is a good instrument in the detection of remediation effect, including the diversity of microbial groups. Application of community-level physiological profiling to bioremediate soils can offer numerous indices on microbial functional activity and diversity, along with the assemblage of functional community (Martínez-Toledo et al., 2024).

The aim of this research was to analyse the structure of reduced functional guilds in soil microbiome after 1 year of bioremediation. Hypotheses related to the aim was formulated as research questions: i) are there still visible effects of historical pollution on functional diversity of microbiome? ii) an in-depth analysis of functional microbiome reveal a

reduced presence of specific functional guilds from the entire community? iii) is there a pattern of functional decrease in community associated with a site and a specific guild?

MATERIALS AND METHODS

The samples used in the research were collected from Baia Mare (Baia Mare County), from 5 sites (Craica - CR), Colonia Topitorilor - CT, Ferneziu - FR, Urbis - URB, Romplumb - ROMP) where phytoremediation was applied one year prior (Pop et al., 2024). The Biolog Ecoplates was used to analyse the functional microbiome, in a time interval of 96 hours. Each of the 3 replications from each site was 10^{-4} times diluted, and readings were performed with a spectrophotometer at 590 nm, every 24 hours. From all the samples, an average standard of activity was constructed, to which the standard deviation (+/-) was applied to set the local limits of the functional ecological niche.

The analysis of the results followed the DEMSA (Detailed Exploration of Microbial Sociological Assemblage) model (Stoian et al., 2022). All the readings from each site were compared with the standard and two matrices were built. The first matrix contained all the values that were between the average and the minimum activity of the standard, and this matrix was considered the Narrowing segment of the functional microbiome. The data that were below the minimum standard were considered as a part of the Contraction matrix. All substrates were considered as a functional group, while chemical similar substrates were considered as a functional guild. The 5 functional guilds followed in the research protocol were: Carbohydrates (CH), Polymers (P), Carboxylic & acetic acids (CX), Amino acids (AA), Amines/amides (AM).

Data analysis was performed in RStudio (R Core Team, 2024), with basic statistics extracted from "psych" package (Revelle, 2024) and the differences between sites were assessed with ANOVA and LSD test from "agricolae" package (de Mendiburu, 2023). For each of the matrices used, diversity indices and NMDS ordination ("vegan" package, Oksanen et al., 2022) were extracted to analyse the stability of site-specific functional microbiomes and their potential reduction pattern. Additionally, the correlations between parameters were analysed with "Hmisc" package (Harrell, 2025).

RESULTS AND DISCUSSIONS

The narrowing matrix showed a variety of activities and diversity but not present in all sites (Table 1). This phenomenon was caused by site-specific soil conditions, which favoured specific activities. CH guild is absent in CT sites, but present extremely high values in ROMP and URB. Both sites present a potential reduction of CH functional activity, which is associated with ten different functional groups. For this guild, CT sites present conditions that enable the survival of CH groups, and near to this condition FR is set to almost zero. For P functional guild, CT and FR sites offers the conditions for microbiome survival, both sites being absent from the constraining matrix. In terms of CX guild, the same two sites present a limiting activity near to zero, while for ROMP and URB the narrowing is set to more than 4.5 units. An interesting case was observed for CR site, where CX guild have a restrictive potential of 1.5, while CX of only near to 1 unit. The same site offers the conditions for the survival of AA and AM guilds, with less than 1 unit narrowing of activities. On the other hand, both ROMP and URB act to reduce the AA activity in a similar manner, while for AM was observed a 20% difference.

In terms of the total potential sum of narrowing, ROMP is the most unfavourable site, with 16 units, and followed by URB (Table 1). Both sites act to drastically reduce the potential functional activities. Compared to these conditions, for CT and FR the reducing of activities is set to less than 1 unit, which correspond to less restrictive conditions of both sites.

By analysing the index of confining, a different point of view is set toward the population of the communities that reduce their activities (Table 1). ROMP is responsible for the almost complete change of microbial community. This high value of narrowing index can be explained by the lack of pattern in the reduction of functional activity at microbiome scale. Also, this indicate that several functional groups are more sensitive to soil conditions and reduce

their activities at a larger scale. URB site produce a narrowing index of more than 58%, which explain a change in the initial functional pattern of more than half of community. For both CT and FR sites the values of narrowing are less than 2%, which means a negligible effect for the reduction of a large share of community.

In terms of diversity, the analysis of site-specific microbiomes showed high differences in terms of both community groups and the effect of soil conditions (Table 1). Shannon diversity was set to more than 3 (URB and ROMP), a value which indicate a strong effect on the functional microbiome and that numerous functional groups are reducing their activities. An interesting case was recorded for CT and CR sites, where this index values were lower, which indicates the reduction of activity for very specific functional groups.

Table 1. Microbial functional guilds and diversity in Narrowing range of activities

Sites	CH	P	CX	AA	AM
CR	1.51±0.54bc	0.89±0.24b	1.03±0.47b	0.84±0.34b	0.76±0.14a
CT			0.04±0.04b	0.34±0.34b	
FR	0.35±0.09c		0.29±0.14b	0.10±0.10b	0.02±0.02b
ROMP	4.96±0.40a	1.89±0.35a	5.09±0.66a	3.32±0.56a	0.77±0.38a
URB	3.10±1.00b	1.18±0.22b	4.58±0.48a	3.19±0.35a	0.97±0.11a
Sites	Sum	Index of	Shannon	Simpson	Pielou
CR	5.06±1.08b	12.6±3.45b	2.54±0.16b	0.91±0.01a	0.94±0.00a
CT	0.39±0.32c	0.73±0.60b	0.27±0.19d	0.51±0.26b	0.39±0.28b
FR	0.78±0.15bc	1.51±0.29b	1.25±0.12c	0.68±0.04ab	0.92±0.01a
ROMP	16.0±1.94a	98.8±24.5a	3.31±0.05a	0.96±0.00a	0.97±0.00a
URB	13.0±2.10a	58.1±15.6a	3.15±0.13a	0.95±0.00a	0.94±0.01a

Note: values ± s.e. followed by different letters present significant differences according to LSD ($p<0.05$). Legend: sites - Craica (CR), Colonia Topitorilor (CT), Ferneziu (FR), Urbis (URB), Romplumb (ROMP); guilds - Carbohydrates (CH), Polymers (P), Carboxylic & acetic acids (CX), Amino acids (AA), Amines/amides (AM); Index of - Index of Narrowing.

Simpson index values indicate a medium heterogeneity for these two sites, and less groups that are reducing their activity (Table 1). In contrast, all the other three sites act for the reduction of activities for larger functional groups. These trends are sustained by Pielou`s index, where almost balanced share of communities is reducing their activities, apart from CT site where the narrowing affects differently microbial groups.

The contraction potential of microbial communities is visible in only 3 sites (Table 2), and only for CX guild. The rest of the sites show a variable contraction potential, with a maximum in ROMP for CX guild. All the rest of the guilds show a contraction potential of less than 1 unit of their activities, which indicates that even their metabolic activities are reduced due to the soil conditions there is no possible disappearance from the community.

In terms of contraction index, the highest value was recorded in ROMP site, where the community that contract its activities is 26% different from the average pattern (Table 2). Both CR and URB sites showed less than 6% contraction index values.

Shannon present index a powerful effect of contraction in ROMP community contraction, compared to CR site where the effect was considered negligible (Table 2). This effect is visible in the value of Simpson index, with a heterogeneous community that contract their activities due to soil conditions. At the opposite, for both CR and URB sites, the contraction is visible in a moderate homogeneous community. Pielou`s index present specific functional shares of the microbiomes that reduce drastically their activities in CR site, while the contraction is visible in almost the entire community for the ROMP and URB sites.

Table 2. Microbial functional guilds and diversity in Contraction range of activities

Sites	CH	P	CX	AA	AM
CR			0.03±0.03b		
ROMP	0.81±0.26a	0.37±0.16a	1.90±0.38a	0.82±0.35a	0.22±0.11
URB	0.18±0.10b	0.02±0.02b	0.45±0.24b	0.47±0.25a	0.02±0.02
Sites	Sum	Index of	Shannon	Simpson	Pielou
CR	0.04±0.04b	0.12±0.12b	0.19±0.19c	0.75±0.24a	0.13±0.13b
ROMP	4.14±0.88a	26.1±8.24a	2.77±0.18a	0.92±0.01a	0.90±0.00a
URB	1.15±0.63b	5.63±3.36b	1.59±0.54b	0.68±0.17a	0.85±0.05a

Note: values ± s.e. followed by different letters present significant differences according to LSD ($p<0.05$). Legend: sites - Craica (CR), Urbis (URB), Romplumb (ROMP); guilds - Carbohydrates (CH), Polymers (P), Carboxylic & acetic acids (CX), Amino acids (AA), Amines/amides (AM); Index of - Index of Contraction.

The projection on NMDS ordination for the limiting segment of functional microbiomes show a different stability of communities (Figure 1). Both CT and FR sites are placed in the same area of the ordination and the plots are near one to each other, indicating stable communities and a similar reduction pattern. On the other hand, CR site show a medium dispersion of plot-specific communities, and a position in the -+ quadrat, near to the CT and Cr sites. Both ROMP and URB are placed in different quadrats and are defined by a great dispersion of their communities.

An interesting aspect is that each of these microbiomes are associated with specific

functional guilds, that are responsible for major changes inside the assemblage of the community (Figure 1). CX and AA guilds are very significant in the changes of URB community, and additionally the AM variability can produce distinct changes. For ROMP, a part of the communities is associated with the variability and changes in CH and P guilds, but not completely correlated one with each other. The other two communities are associated with the sum of potential restriction and the index of narrowing, that will modify both the community consortium and the loss of a part of activities.

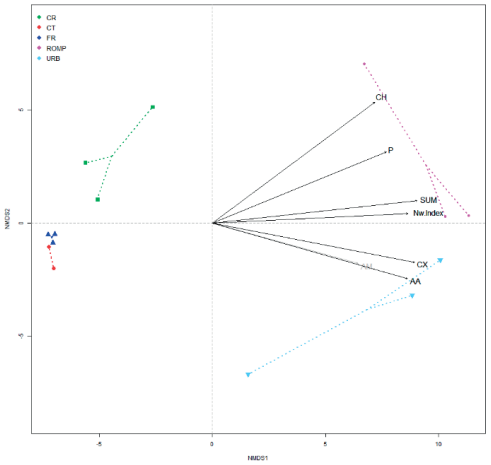


Figure 1. NMDS projection of microbial communities in Narrowed niche. Legend: sites - Craica (CR), Colonia Topitorilor (CT), Ferneziu (FR), Urbis (URB), Romplumb (ROMP); Nw.Index - Index of narrowing; H - Shannon index; S - Simpson index; guilds - Carbohydrates (CH), Polymers (P), Carboxylic & acetic acids (CX), Amino acids (AA), Amines/amides (AM)

In term of contraction potential, CR site present an almost identical community pattern to one URB site (Figure 2). This similarity of contractions indicates a similar microbial assemblage pattern that overlap in both sites. Both ROMP and URB show a high dispersion in on the ordination graph. URB dispersion is oriented parallel with axis 1 of ordination and is not associated with a specific functional guild activity.

The ROMP site-specific communities are present in three quadrats of the ordination, associated with the contraction of different groups activities (Figure 2). The upper point of this site is placed in -+ quadrat, and AA guild vector is oriented toward it. The middle point is placed in the -- quadrat and is more related to the sum of activities that are contracting within the community. Between these two points are placed the P activity guild, which indicate a different contraction scenario based on the contraction of this guild. This scenario is the most related to the index of contraction and that can completely modify the structure of the community. The last point is placed in the +- quadrat but is not related to any of the guild's activities. Between this and the second point, the presence of CX vector indicate another contraction scenario that is related to the drastic reduction of this guild activity.

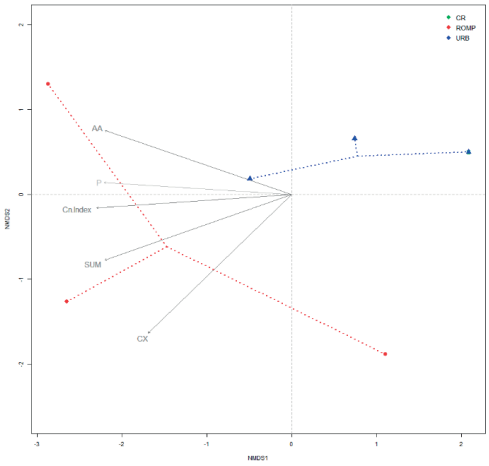


Figure 2. NMDS projection of microbial communities in Expansion niche. Legend: sites - Craica (CR), Urbis (URB), Romplumb (ROMP);Cn.Index - Index of contraction; H - Shannon index; S - Simpson index; guilds - Carboxylic & acetic acids (CX), Amino acids (AA)

The NMDS projections for narrowing and contraction of microbiome showed a great impact of specific functional guilds. The in-depth exploration of functional group relation with community projections shows a gradual reduction from restriction to contraction in niche (Table 3). Most of the CH and CX groups are present in the selective community, which indicate a sensitivity to pollution of these microbiome components. Their impact in the stability of communities range from very to distinct (***/**). The same phenomenon was observed for P and AA groups, indicating that a complex segment of microbiome is narrowing due to the soil conditions. AM2 group show a

significant presence in the reduction of microbial activity, being the only group from AM guild that show sensitivity to soil conditions.

The contraction observed in microbial communities relies on a reduced number of functional groups, indicating a high sensitivity to soil pollution (Table 3). For the three sites that show this type of extreme activity reduction, most of the functional groups belong to CX substrate category. Their impact in the community ranges from significant to distinct (CX7), with a total of 6 functional groups in this category. Both CH and P are rare in the contracted community, with an overall significant impact in the assemblage. On the other hand, the most present set of groups belong to AA guild, with only AA2 that present a significant impact in the contraction status. An interesting case is observed for AM1, which have a significant share in the contracted community.

Table 3. Microbial functional groups associated with Intensification and Expansion in the range of activities

Niche segement	Group	Sig	Group	Sig	Group	Sig
Narrowing	CH1	***	CX1	**	P1	***
	CH2	**	CX2	***	P2	**
	CH3	**	CX3	****	P3	**
	CH4	**	CX4	***	P4	**
	CH5	***	CX5	**	AA1	***
	CH7	**	CX6	***	AA2	***
	CH8	**	CX7	**	AA3	**
	CH9	***	CX8	***	AA4	**
	CH10	***	CX9	**	AA5	***
					AA6	***
					AM2	*
Contraction	CH4	*	CX2	*	P1	*
	CH9	*	CX4	*	AA1	*
			CX6	*	AA2	**
			CX7	**	AA3	*
			CX8	*	AA6	*
			CX9	*	AM1	*

Note: groups by * significant impact in NMDS ordination (*p<0.05*; p<0.01**; p<0.001* ***). Legend: functional guilds - CH1 - Pyruvic acid methyl ester, CH2 - d-Cellobiose, CH3 - α-d-Lactose, CH4 - β-Methyl-d-glucoside, CH5 - d-Xylose, CH7 - d-Mannitol, CH8 - N-Acetyl-d-glucosamine, CH9 - Glucose-1-phosphate, CH10 - d,l-α-Glycerol phosphate, CX1 - d-Glucosaminic acid, CX2 - d-Galactonic acid γ-lactone, CX3 - d-Galacturonic acid, CX4 - 2-Hydroxy benzoic acid, CX5 - 4-Hydroxy benzoic acid, CX6 - γ-Hydroxy butyric acid, CX7 - Itaconic acid, CX8 - α-Keto butyric acid, CX9 - d-Malic acid, P1 - Tween 40, P2 - Tween 80, P3 - α-Cyclodextrin, P4 - Glycogen, AA1 - l-Arginine, AA2 - l-Asparagine, AA3 - l-Phenylalanine, AA4 - l-Serine, AA5 - l-Threonine, AA6 - Glycyl-l-glutamic acid, AM1 - Phenylethylamine, AM2 - Putrescine.

The reduction of functional activity is observed as the interaction between the different functional guilds (Table 4), that shape the structure of communities. The narrowing at community level implies a significant positive correlation between all functional guilds, indicating large coordinate process. The highest correlation was observed between CX and AA guilds, both presenting numerous groups in the narrowed niche. The AM is the less associated with the rest of functional guilds, indicating a more independent activity of this microbiome in terms of the other ones. Based on their coupled activity, CX and AA guilds present an important share in the narrowing of the total sum of activities. These values indicate that any restriction of activities in these two groups will impact the total activity almost completely. Both P and CH guilds are identically correlated with the sum of activities, which indicate that any of these guilds can be used to trace the narrowing of the activity. Related to the index of alteration, the sum of activities has the most important role, the high value of correlation indicating a potential change in the pattern of microbiome groups that reduce their presence and functionality. All guilds present correlation values with Shannon diversity between 0.75-0.85, sustaining that a complex microbiome, from different guilds, narrow their activities, but at different levels. The shift between heterogenous and homogeneous of the selective communities is associated with all the guilds at values under 0.60, an aspect that sustain that a great change within a community will be produced only when numerous guilds will reduce at once their activities.

Table 4. Interrelations between microbial guilds and indices in Narrowing and Contraction niche

									Narrowing
	P	CH	CX	AA	AM	Sum	In	H	S
P		0.86	0.90	0.87	0.84	0.94	0.92	0.85	0.58
CH	0.71		0.88	0.87	0.64	0.94	0.90	0.84	0.56
CX	0.87	0.74		0.97	0.78	0.98	0.93	0.85	0.55
AA	0.87	0.74	0.84		0.80	0.97	0.92	0.83	0.52
AM	0.96	0.56	0.90	0.84		0.81	0.74	0.81	0.57
Sum	0.93	0.84	0.97	0.93	0.90		0.96	0.88	0.58
In	0.97	0.82	0.93	0.93	0.93	0.99		0.75	*0.48*
H	0.74	0.83	0.91	0.88	0.72	0.93	0.87		0.69
S	*0.45*	0.55	0.60	0.59	*0.45*	0.61	0.56	0.68	
Contraction									

Note: values in *italic* did not present significant correlations (*p<0.05*). Legend In - Index of alteration; H - Shannon index; S - Simpson index; guilds - Carbohydrates (CH), Polymers (P), Carboxylic & acetic acids (CX), Amino acids (AA), Amines/amides (AM).

The contraction correlations present lower values and a lower association potential between guilds and parameters (Table 4). In terms of this niche segment, AM is significant associated with P and CX, all three of them being highly correlated with the sum of

activities that are extremely reduced. An interesting case was observed for AA, which have a lower association potential with the other guilds but present a high importance in the total sum of activities. This makes AA a good candidate for the detection of contraction phenomena, even when small changes are visible within this guild. The index of alteration is almost completely determined by the total activity, indicating that contraction process will produce high imbalances in the share that each group have in the community. The changes in P activities are the most important in the assemblage of the contracted communities, while CX, AA and AM changes will act in a similar way. The CX guild is the most important type of microbiome that affect the diversity in contracted community, affecting at the same time the homogeneity.

CONCLUSIONS

The narrowing and contraction potential in functional communities is visible in the low scores recorded for all the guilds analysed, resulting in potential losses of high shares of the total activities.

The index of narrowing recorded almost 99% for Romplumb site, and an extra alteration of 26.1% in the contraction segment of ecological niche.

For both the narrowing and contraction segments of functional niche, the diversity indices present fluctuating values and indicate a high impact of pollution.

The narrowing phenomenon is visible in two different scenarios, with the maintenance of community stability and response (Colonia Topitorilor and Ferneziu), or with a plot-specific response and reduced community stability (Romplumb and Urbis).

Contraction phenomena lead to the high reduction of multiple functional groups activities, the lack of community stability and multiple scenarios of future functional assemblages.

In terms of narrowing, most of the groups can influence the development of different functional assemblages based on one-to-one positive interactions.

The contraction phenomenon is accelerated by the separate interaction between Amines/amides with Polymers and Carboxylic & acetic acids guilds.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

ASSESSMENT OF MICRO AND MACROELEMENTS UPTAKE IN MAIZE PLANTS IN RESPONSE TO DIFFERENT NITROGEN AND PHOSPHORUS FERTILIZATION RATES

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Abstract

This paper aims to evaluate the effects of nitrogen and phosphorus mineral fertilization, applied in varying doses, on the quality of maize crops grown in acidic soils. The study is based on a long-term bifactorial experimental design, involving progressively increasing doses of nitrogen and phosphorus (arranged in a 5P × 5N scheme, with N_0-N_{160} combined with P_0, P_{40}, P_{80}, P_{120}, and P_{160}), alongside lime application. The findings offer valuable insights into the long-term impacts of these fertilization practices on crop quality. Results show that long-term fertilization combined with calcic amendments induces statistically significant changes in the macroelements content of maize leaves specifically nitrogen, phosphorus, potassium, calcium, and magnesium and the microelements content, including copper, zinc, manganese, and iron.

Key words*: acidic soil, maize, mineral fertilization.*

INTRODUCTION

Maize is one of the most important cereal crops in the world, alongside wheat and rice, serving as food for both humans and animals and as a primary feedstock for biogas production (Singh et al., 2024).

Currently, in the context of global warming and population growth, it becomes increasingly important to obtain high-quality crops (Bojtor et al., 2021). In this regard, soil quality must be considered, as soil acidity and nutrient availability are among the primary factors limiting crop growth (Gondek & Mierzwa-Hersztek, 2023).

To achieve better harvests, it is essential to determine the plant's chemical composition as a basis for fertilizer application (Kalra, 1998).

Assessing the nutritional status of maize plants under different nitrogen and phosphorus application rates provides insight into fertilizer use efficiency (BąK et al., 2016; Pasley et al., 2019).

In Romania, acidification and excessive moisture are the most pronounced degradation processes of clay-illuvial soils in north-western Transylvania. Pedological and agrochemical studies conducted in the area indicate that approximately 60% of agricultural land exhibits an acidic reaction. Soil acidity is a natural phenomenon in regions with an excess water regime (Kurtinecz, 2010). As a result, since autumn 1961, it has been considered a major imperative to establish long-term experiences located on the acidic soils of Livada Agricultural Research Station (SCDA Livada).

These experiments offer the possibility to monitor changes in soil characteristics under the effects of limestone and fertilization amendments and how these changes are reflected in the quantity and quality of crops. The nutritional status of a plant can be evaluated by measuring its total nutrient content relative to its dry matter.

The objective of this study is to evaluate the uptake of essential macronutrients, including nitrogen, phosphorus, potassium, calcium, and magnesium, as well as the absorption of micronutrients such as copper, zinc, manganese, and iron, under the influence of nitrogen and phosphorus mineral fertilization. The concentration of an element is directly

influenced by its presence in the soil and its interactions with other micro or macroelements.

MATERIALS AND METHODS

The research presented in this paper was carried out on one of the representative soils of north-western Transylvania, specifically on a luvic brown soil.

The experimental field is in the central part of the area occupied by clay-illuvial soils, at SCDA Livada, Satu Mare County, within a stationary experimental setup (Figure 1).

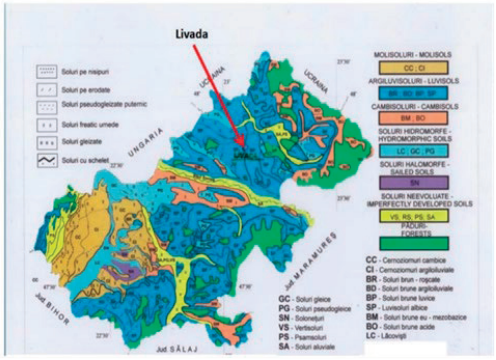


Figure 1. Soil map of Satu Mare County (Asvadurov&Boeriu, 1983)

Experiments on the amendment and fertilization of acidic soils at SCDA Livada were initiated in 1961, with a grain rotation system in place. In 2022, maize was sown (Figure 2).



Figure 2. Maize Field View – SCDA Livada

In the long-term experiment conducted at SCDA Livada, mineral fertilization was applied annually, with lime treatments performed every 4-6 years. The experiment was bifactorial, evaluating the effect of phosphorus and nitrogen fertilization (with five progressively increasing dose levels each, in a 5P × 5N design, based on P_0, P_{40}, P_{80}, P_{120}, P_{160}) on the quality of maize plants.

Seventy-five samples of maize leaves were harvested and analysed for their macro- and micronutrient composition.

After removing impurities and washing, the plant material is dried initially on filter paper at room temperature and subsequently in an oven with ventilation at 70°C. The leaves were ground to pass through a 2 mm sieve.

Total nitrogen content was determined by a wet digestion procedure using sulfuric acid, followed by sodium hydroxide distillation, and sulfuric acid titration (Kjeldahl method).

The plant samples were mineralized with a mixture of nitric, perchloric, and sulfuric acid followed by colorimetric determination of total phosphorus content at a spectrophotometer with ammonium vanadate-molybdate solution (Răuță & Chiriac, 1980).

In the same extract, potassium content was determined at the flame photometer, and those of calcium, magnesium, copper, zinc, manganese, and iron were determined by atomic absorption.

The results were analysed using variance analysis (ANOVA), followed by Tukey's interval test for multiple comparisons between all mean values (Ceapoiu, 1968). For all graphical representations, means followed by the same letter do not differ significantly at the 5% level ($P<0.05$) according to Tukey's HSD test.

RESULTS AND DISCUSSIONS

For harmonious development, maize plants require specific minerals at different growth stages. Therefore, determining the content of macroelements such as nitrogen, phosphorus, potassium, calcium, and magnesium in various plant organs can indicate the optimal doses of mineral fertilizers needed to achieve the best possible yields in corn crops (Gaj et al., 2020).

As is well known, inorganic fertilizers rapidly release the nutrients that plants require (Makinde & Ayoola, 2010). This fact can be easily observed by analysing the nutrient content

of plants harvested from soils fertilized with these products and the absence of visible symptoms of nutrient deficiencies.

The nitrogen in ammonium nitrate, a commonly used nitrogen fertilizer, is present in both ammonium and nitrate forms. While the nitrate form is readily available for plant absorption, the ammonium form is quickly converted into nitrate by soil bacteria through the nitrification process and it becomes available for plant absorption. After absorption, nitrogen is transported into the leaves where it contributes to photosynthesis, which helps the growth and development of maize plants (Wierzbowska et al., 2022)

Chemical analyses of maize plants harvested from the experimental field revealed that increasing doses of nitrogen fertilization led to statistically significant changes in the average nitrogen content of maize leaves. A statistically significant increase was observed starting from the N_{100} dose. The mean nitrogen content ranged from 1.78% in the N_{50} fertilized variant to 3.33% in the N_{200} fertilized variant (Figure 3).

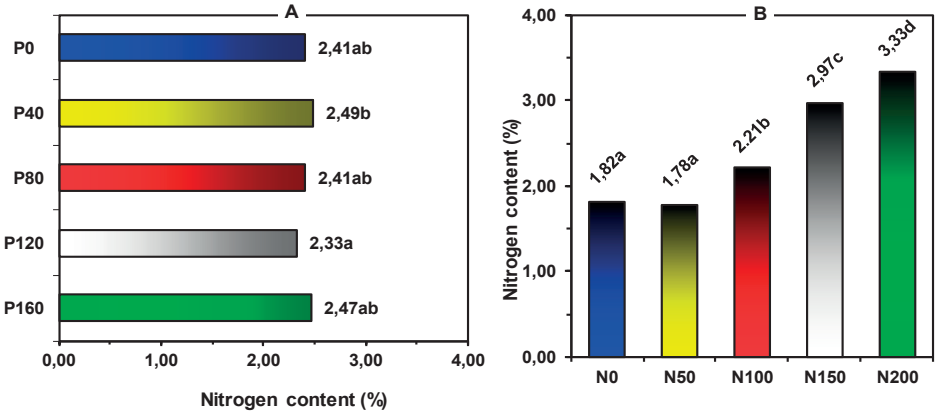


Figure 3. Effects of Phosphorus (A) and Nitrogen (B) Fertilization on Nitrogen Content in Maize Leaves

Phosphorus fertilization and nitrogen application enhance root system development and facilitate better nitrogen absorption (Graciano et al., 2006; Blandino et al. 2022). Conversely, an adequate phosphorus level promotes the formation of adenosine triphosphate (ATP), which plays a crucial role in nitrogen metabolism (Khan et al., 2023). Considering these aspects, the P_{40} dose led to a statistically significant increase in nitrogen content in this study.

Phosphorus plays a crucial role in nutrient storage and transport within plants, but its absorption is influenced by soil pH and the presence of compounds that can immobilize phosphate ions.

Alley et al. (2019) state that due to the low mobility of phosphorus in the soil, applying phosphorus fertilizers can increase the amount of phosphorus available to plants.

The analysis of the chemical characteristics of maize leaves indicated that nitrogen fertilization caused statistically significant changes in the average phosphorus content of maize plants compared to the control (Figure 4).

Phosphorus levels decreased from 0.47% in the N_0 variant to 0.26% in the variant with the highest nitrogen dose, likely due to soil acidification caused by increased nitrogen application, which reduced phosphorus absorption in the plants (Koper & Lemanowicz, 2008). In contrast, increasing phosphorus doses resulted in a higher average phosphorus content in leaves compared to the unfertilized variant for P_{80}, P_{120}, and P_{160}, with statistically significant differences.

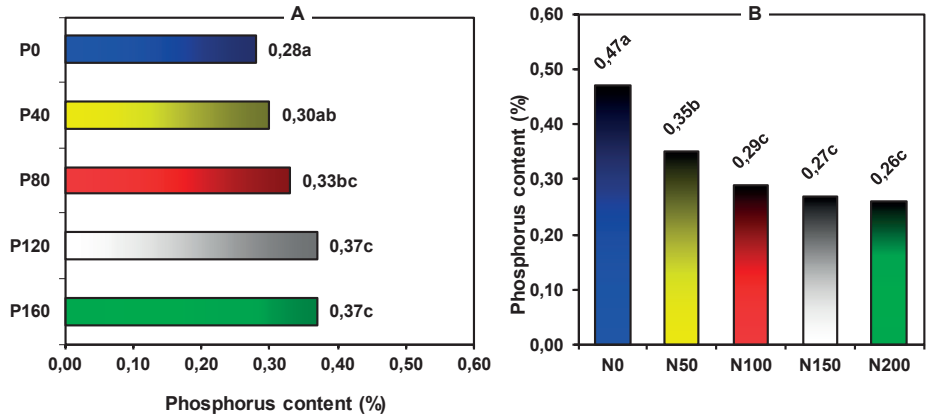


Figure 4. Effects of Phosphorus (A) and Nitrogen (B) Fertilization on Phosphorus Content in Maize Leaves

Although the other phosphorus fertilizer doses increased mean phosphorus levels compared to the control, these changes were not statistically significant.

Potassium, along with nitrogen and phosphorus, is one of the essential macronutrients for plants, playing a crucial role in plant metabolism and enhancing their resistance to water stress, diseases, and pests (BąK et al., 2016).

The influence of phosphorus fertilization on the average potassium content in maize leaves decreased compared to the control as the fertilizer dose increased (Figure 5).

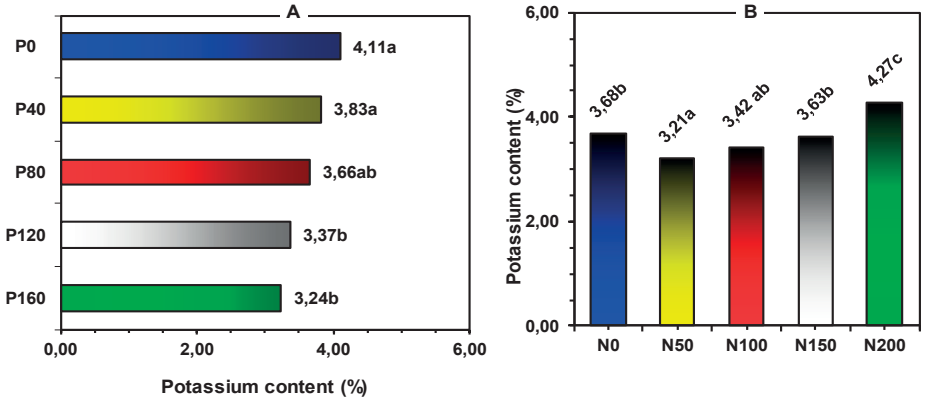


Figure 5. Effects of Phosphorus (A) and Nitrogen (B) Fertilization on Potassium Content in Maize Leaves

The low bioavailability is due to calcium and magnesium ions being present, and not potassium being included in the fertilization formula used in the experiment (Kalra, 1998; Malakshahi Kurdestani et al., 2024). The determined potassium (K) content falls within the range of 3.0-4.0% K, which is considered

After applying the highest nitrogen dose, maize leaves had a significantly higher potassium content than unfertilized and other fertilized varieties. The application of high doses of sufficient for the seedling stage according to Đalović et al. (2020). Fertilization with increasing nitrogen doses led to lower average potassium content values than the control variant, possibly due to the dilution effect stemming from the increase in dry biomass in the fertilized variants (Nenova et al., 2019)

nitrogen may increase nutritional demand, stimulating potassium absorption. Nitrogen positively influences root system development and boosts metabolic processes, leading to a

higher rate of potassium absorption from the soil (Pasley et al., 2019).

Calcium is an element that supports the development of plants, having an important role in protecting plants from the strong sun rays; calcium regulates osmotic and ionic processes in cell membranes (Gaj et al., 2018).

In this study, maize was cultivated on moderately to strongly acidic soil (pH 5.2), which resulted in a low Ca and Mg content in an antagonistic relationship between K (Figure 6). The application of phosphorus fertilization resulted in a slight increase in the average calcium content values of the analysed samples, despite all fertilization variants showing low calcium levels ranging between 0.11% and 0.23% (Răuță, & Chiriac; 1980, Kelling et al., 2000).

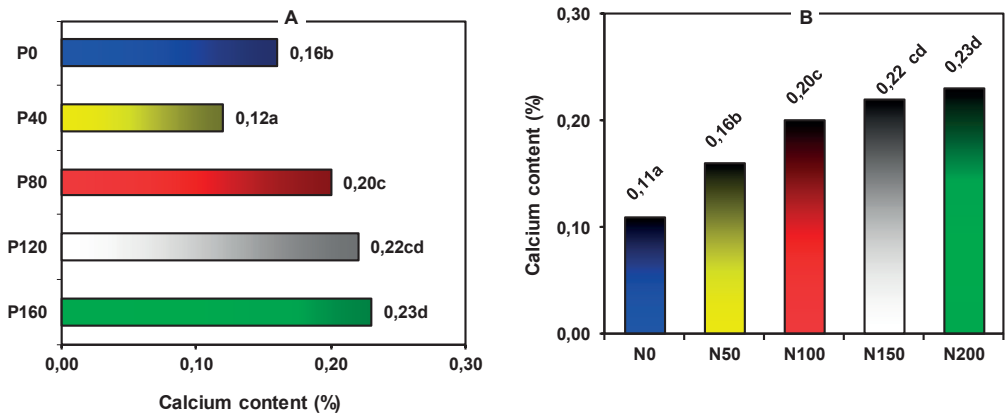


Figure 6. Effects of Phosphorus (A) and Nitrogen (B) Fertilization on Calcium Content in Maize Leaves

Nitrogen fertilization stimulates root development and increases transpiration, facilitating the absorption and transport of calcium into the leaves from the first dose of applied nitrogen. However, nitrogen fertilization did not increase the calcium content in the leaves beyond low levels.

Magnesium, an essential component of chlorophyll, is a vital nutrient for plant development. It plays a key role in photosynthesis, contributing to higher yield (Gaj et al., 2018). The magnesium content was in the same range of low values as the calcium content (Figure 7).

Long-term fertilization and calcium amendments induce statistically significant changes in micronutrient contents.

Copper, a micronutrient that supports plant defence mechanisms and photosynthesis, was one of the chemical characteristics evaluated in maize plants harvested from the experimental field. Increasing doses of phosphorus fertilization led to copper immobilization, reducing its bioavailability for maize plants. As a result, the average copper content in maize leaves ranged between 5.9 mg/kg and 6.9 mg/kg, which falls within the normal concentration range (Figure 8). On the other hand, the reduction of Cu concentration could be partly explained by a decrease in the colonization of roots by arbuscular mycorrhizal fungi (AMF) due to increasing P application (Zhang et al., 2020). Nitrogen fertilization has caused increased copper mobility in the soil, making it more accessible to maize roots. Plants have a more intensive metabolic rate at high nitrogen levels and demand more micronutrients, including copper.

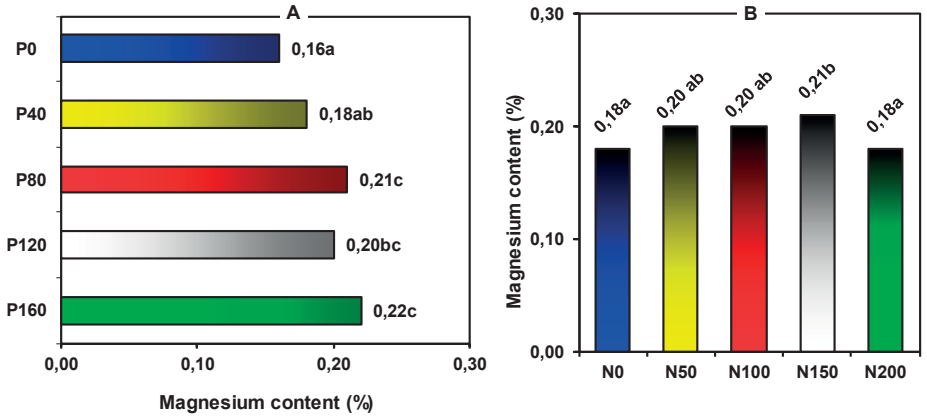


Figure 7. Effects of Phosphorus (A) and Nitrogen (B) Fertilization on Magnesium Content in Maize Leaves

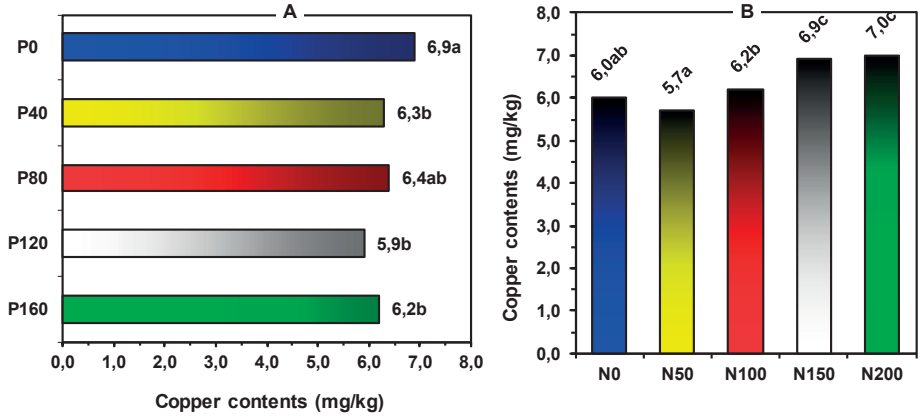


Figure 8. Effects of Phosphorus (A) and Nitrogen (B) Fertilization on Copper Content in Maize Leaves

This may explain the statistically assured increases in mean copper content values in maize plants.

The highest values of 6.9 mg/kg and 7.0 mg/kg were observed at high nitrogen fertilization rates, N_{150} and N_{200}, respectively.

Iron, which is absorbed into plants by the root system, supports photosynthesis and contributes to increased nitrogen uptake by the plant. The iron content values in maize samples indicate the same blocking effect following fertilization with different doses of phosphorus as for copper. Nitrogen fertilization decreased the average iron content in maize leaves, with the lowest values obtained in N_{100} and N_{150} fertilized variants. These changes are statistically assured against the control (Figure 9). The explanation could be due to the dilution effect induced by biomass expansion or the fact that nitrogen in large quantities can influence the balance of nutrient absorption. Fe competes with transition metals such as Cu, Zn, and Mn in its uptake, transport, and chemical reaction with plant cells (Rai et al., 2021). A higher copper supply may limit iron availability. Zinc plays a key role in calcium transport within plants, supporting the development of leaves and grains. Its concentration in maize leaves follows a decreasing trend with increasing phosphorus application, with significant reductions observed at high P_{120} and P_{160} doses. These changes are statistically assured (Figure 10).

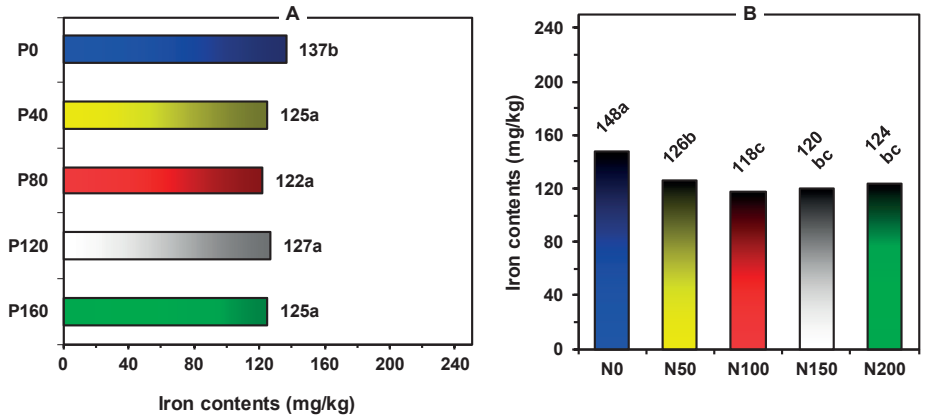


Figure 9. Effects of Phosphorus (A) and Nitrogen (B) Fertilization on Iron Content in Maize Leaves

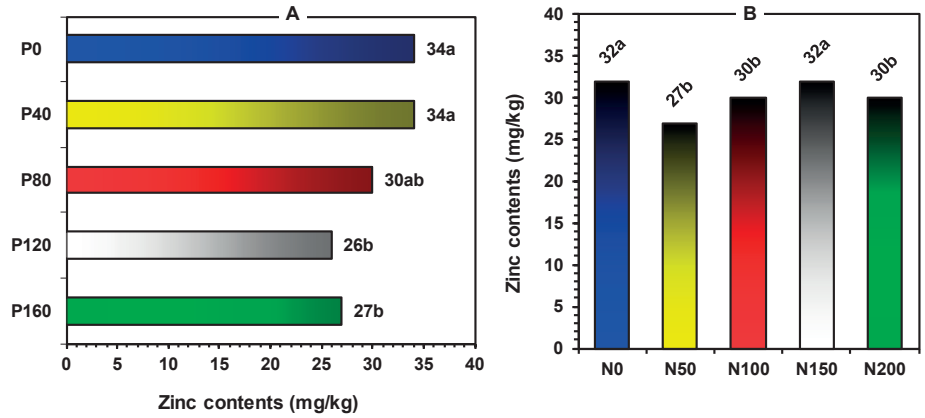


Figure10. Effects of Phosphorus (A) and Nitrogen (B) Fertilization on Zinc Content in Maize Leaves

Moraghan (1985) reported that Zinc deficiencies induced by phosphorus in plants typically result from plant dilution effects, limited zinc translocation, or an imbalance between phosphorus and zinc.

Nitrogen fertilization has resulted in variations in mean zinc content ranging from 27 mg/kg to 32 mg/kg, which fall within the range of normal concentrations for maize plants (Răuță & Chiriac, 1980). Manganese plays an important role in enzyme activity, facilitating nitrogen assimilation. Manganese contents found in maize plants indicated inhibited absorption of this micronutrient, with the effect becoming more pronounced as the applied phosphorus dose increased (Figure 11).

These changes are statistically assured compared to the variant without phosphorus fertilization.

Fertilization with increasing doses of nitrogen caused an increasing trend of manganese content in the analysed samples. Because maize can accumulate large quantities of heavy metals, nitrogen can influence the balance of other nutrients, reducing competition between manganese and other metals, such as iron or zinc, and allowing for more efficient manganese absorption (Wyszkowski & Brodowska, 2021).

The acidification caused by nitrogen fertilization favours manganese absorption in the plant, leading to statistically significant increases compared to the control and between variants, starting from the N_{80} dose. Micronutrients are required in smaller quantities than macronutrients for plant growth and development, but this does not diminish their importance in plant nutrition (Arnon & Stout, 1939; Johnson & Mirza, 2020).

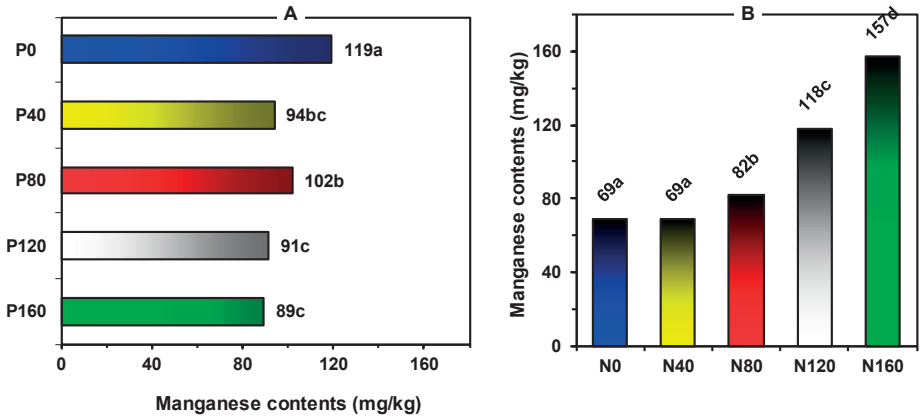


Figure 11. Effects of Phosphorus (A) and Nitrogen (B) Fertilization on Manganese Content in Maize Leaves

A deficiency in any essential element can negatively impact crop yields, making it crucial to determine the optimal fertilization formula to prevent any nutrient deficiencies.

The nutritional status of plants, reflected in their macro- and micronutrient content, can be influenced not only by nutrient deficiencies or excesses but also by a complex interplay of environmental factors such as water availability, climate, and other conditions (Houmani & Corpas, 2024).

CONCLUSIONS

The application of phosphorus had a favourable effect on nitrogen valorisation due to the stimulation of the development of the root system. Following fertilization with increasing doses of phosphorus, increases in nitrogen, phosphorus, calcium, and magnesium contents were observed from the analysed maize plants.

Dose fractionation of nitrogen predicted the depressive effect of excessive nitrogen doses such as inhibiting the absorption of other nutrients (e.g., potassium, calcium, magnesium, copper, manganese) due to nutritional imbalances or the decrease in the efficiency of nitrogen use through leaching losses.

Upon applying progressively increasing nitrogen doses, statistically significant increases were recorded in the concentrations of nitrogen, potassium, calcium, and copper in maize leaves compared to the control variant. Calcium amendment and long-term fertilization induce changes in the chemical composition of maize plants, reflecting the cumulative effect of previous treatments. Therefore, interpreting these results in the context of climate change is valuable for identifying the optimal fertilizer doses required for sustainable crop management.

ACKNOWLEDGEMENTS

This research work was carried out with the support of SCDA Livada and funded by the Ministry of Agriculture and Rural Development within Project ADER 1.5.3/2019 entitled: "Assessing the long-term impact of fertilization and amendment on the environment, in the context of global warming, to ensure sustainable management of acidic soil fertility" and the preparation of this paper was funded within Project ADER 18.1.1/2023 entitled: "Developing solutions for restoring soil health in conditions of maintaining environmental sustainability by capitalizing the fertilizing potential of organic fertilizers".

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

SOIL PHOSPHOMONOSESTERASES ACTIVITY AND PHOSPHORUS AVAILABILITY IN DIFFERENT TILLAGE SYSTEMS AND INOCULATIONS WITH *Bacillus megaterium* var. *phosphaticum*

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Abstract

Phosphorus (P) is a crop-limiting nutrient, and its availability is challenging due to low solubility and high fixation rate. Rhizosphere activity, which mediates P uptake, can be promoted by inoculating phosphorus-solubilizing bacteria (PSB). This study aimed to evaluate soil total and accessible phosphorus (TP and AP), acid and alkaline phosphatases activity in response to PSB inoculation in different tillage practices. The field experiment was conducted on winter wheat, cultivated under conventional (CT), no-tillage (NT) and reduced tillage (RT). Both inoculations and the tillage systems were found to be associated with changes in TP and AP. In the CT and RT systems, alkaline phosphatase showed an increase in the bacterial inoculum treatments, 2-31% higher than the control in CT and 15-41% in RT. Across tillage systems, acid phosphatase activity was highest in RT (1.59-1.86 µg p-nitrophenol g^{-1} h^{-1}), followed by NT (1.46-1.72 µg p-nitrophenol g^{-1} h^{-1}) and CT (1.16-1.28 µg p-nitrophenol $g^{-1}h^{-1}$). The soil air-water regime controlled by tillage practices influences rhizosphere activity and, together with bacterial inoculation, leads to variations in the investigated parameters.

Key words*: acid phosphatase, alkaline phosphatase, no-tillage, phosphorus-solubilizing bacteria.*

INTRODUCTION

Phosphorus (P) is a limiting element of crop nutrition, and its management requires particular attention. Even though P is found in large organic (Po) and inorganic (Pi) amounts, it has a low mobility (Milić et al., 2019) and only a small percentage is accessible to plants as HPO_4^{2-} and $H_2PO_4^-$ ions in soil solution. In addition, phosphorus natural sources are non-renewable and declining, making fertilization with large amounts unsustainable and polluting (Prasad and Chakraborty, 2019). P dynamics are regulated by biological and biochemical processes, which depend on soil physical properties, pH, and soil organic matter (SOM) content (Davidescu & Madjar, 2009).

Most biochemical reactions are driven by enzymes, which are sensitive to changes in the soil environment. Enzymatic activity regulates and improves soil ecology, physical-chemical characteristics and, by extension, soil fertility (Das & Varma, 2010; Huang et al., 2023).

Of the enzymes found in soil, phosphatases are vital in the processes of Po mineralization. Acid and alkaline phosphatases originated from microorganisms or plant roots. Their activity involves releasing free phosphates from inorganic fertilizers and catalyzing the breakdown of organic compounds with P (Acosta-Martínez & Tabatabai, 2011).

To improve P mobility and availability to plants, many studies have successfully tested the effectiveness of multiplication and soil fertilization with phosphorus solubilizing bacteria (PSB). *Bacillus* sp. is one of the most common and documented genera with extensive field applications. This is due to its species characterized by high adaptability and complex metabolic functions. Among their mechanisms of actions, we mention the synthesis of phytohormones (Tiwari et al., 2019), antibiotics (Shafi et al., 2017), organic acids and enzymes that enhance crop resistance, promoting growth and productivity (Robles Montoya et al., 2020). Studying the species *Bacillus megaterium*, Ibarra-Galeana et al. (2017) found that it exhibits both alkaline and acid phosphatase activity, thus contributing to enhanced solubilization of organic phosphates.

Considering that mineral fertilization is almost unavoidable, there is a potential to promote the accessibility of P to plants by using integrated crop nutrition formulations with the application of bacterial inoculants (Bargaz et al., 2021). Mixed use of mineral fertilizers with PSB can increase uptake efficiency as well as environmental protection.

Wheat (*Triticum aestivum* L.) is the second most widespread crop in Romania. The cultivated area is 2.2 million ha, with a total production of 9.6 million tons in 2024, ranking fourth in the European Union (INSSE). Achieving high yields is a challenge and involves extreme management measures such as intensive tillage and the use of large amounts of fertilizers, which are also the main causes of environmental degradation in the agricultural sector (Gomiero, 2016; Sun et al., 2024).

Although conventional tillage (CT) maximizes yields, this is often associated with negative effects on soil quality caused by repeated mechanical operations. Conversely, conservative practices create a balance between soil chemical, physical and biological attributes by reducing (minimum tillage) or avoiding soil disturbance (no-tillage) storing a significant quantity of organic matter (SOM) (Szczepanek et al., 2023; Singh et al., 2023). SOM accumulation leads to an abundant and diversified microbial community with a positive impact on soil nutrient cycling and plant uptake (Saini, 2024).

This study aimed to evaluate the enzymatic and chemical response to mineral and biological fertilization of autumn wheat under different management soil systems. We hypothesized that bacterial inoculum together with mineral fertilizer could increase accessible phosphorus in the soil. Another hypothesis was that combined fertilization might increase the intensity of phosphomonoesterases activity, and this could be higher with mulch soil management practices.

MATERIALS AND METHODS

Experimental Site and Treatments

The research was conducted at the Ezăreni Student Research and Practice Station of the "Ion Ionescu de la Brad" Iasi University of Life Sciences (47°7'24.6"N and 27°30'59.76"E). For the field experiment, the growing season of winter wheat from October 2023 to June 2024 was considered. This crop is part of a multiyear rotation along with maize, sunflower and peas. Based on long-term meteorological reports, the experimental field is defined by a temperate continental climate. From sowing to grain harvest, the mean temperature was 9.5°C and the sum of precipitation was about 354.4 mm (Figure 1). The environmental conditions from the time of sowing and during crop growth were favorable. Thus, before the winter season, the crop received the required sum of active temperatures of 500°C. From the end of winter until harvest, both temperatures and rainfall were at optimal levels, so that plant development and ripening proceeded at a normal physiological rate. The recorded climatic data were also favorable for the functioning of *Bacillus megaterium,* although this bacterium is known for its resistance to extreme environmental influences (Biedendieck et al., 2021).

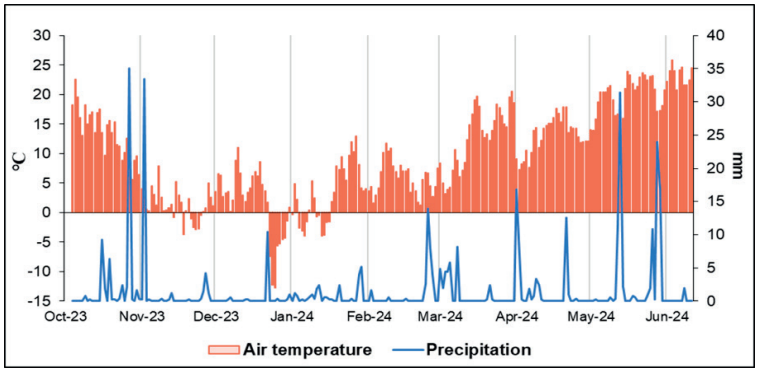


Figure 1. Air temperatures and precipitation from October 2023 to June 2024 (www.fieldclimate.com)

The soil type in the research area is Cambic Chernozem, according to the Romanian Taxonomic Soil System (SRTS, 2012). For a general description of the soil in the experimental field before establishment, the basic the basic soil properties are summarized Table 1.

Table 1. Soil chemical and physical properties

Soil parameter	*Tillage system*		
	CT	**NT**	**RT**
pH	6.2	6.2	6.3
Organic C (%)	2.06	1.94	2.43
Total N (%)	0.178	0.169	0.207
Total P (%)	0.0425	0.0478	0.0463
Available P (ppm)	25	52	51
Texture	Clay-loam (Mihu et al., 2022)		
Bulk density (g/cm^3)	1.14	1.36	1.33

The field experiment was bifactorial and considered the tillage systems and fertilizer treatments described in table 2 and table 3. The local Glosa wheat variety was sown on October 17, 2023 at a row spacing of 17 cm. NP fertilizer was applied at crop sowing over the entire experimental area and the bacterial strain was inoculated manually.

Table 2. Soil tillage systems

Tillage system	*Description*
Conventional (CT)	plowing at 25-30 cm; seedbed preparation at 8-10 cm
No-tillage (NT) with mulch	direct seeding in the last 10 years
Reduced tillage (RT) with mulch	loosening with scarifier at 40 cm, after 9 years of NT

In the CT system, fertilizers were mixed into the soil when preparing the seedbed for sowing. In NT and RT, the biofertilizer was applied to the soil surface and NP fertilizer was inoculated at the same time as the seed. For the second fertilization, Ecofertil was mixed with Corona N for T2, T3 and T4 and sprayed on plants and soil. Ecofertil is a commercial product based on *Bacillus megaterium* var. *phosphaticum*, produced by Antibiotice S.A. (Iasi).

Table 3. Fertilizer treatments

Fertilizer treatment	*Initial fertilization*		*Second fertilization*	
	OCTOBER 17, 2023 (before sowing)		APRIL 01, 2024	
	N_{10} P_{24} (200 kg ha^{-1})	**PSB (Ecofertil - 1× 10^{11} CFU)** (15 l ha^{-1} year^{-1})		**Corona N (21%)** (3 kg ha^{-1})
T0 (control)	+	-	-	-
T1	+	**100 %**	**100 %**	-
T2	+	**75 %**	**75 %**	+
T3	+	**100 %**	**100 %**	+
T4	+	**125 %**	**125 %**	+

Note: PSB - Phosphorus Solubilizing Bacteria; CFU - Colony Forming Unit; "+" means that the listed fertilizer has been applied; "-" means that the listed fertilizer has not been applied; % of PSB means the fraction of the manufacturer's recommended rate.

Soil Sampling and Analysis Methods

Soil sampling was conducted considering the analysis to be performed: acid and alkaline phosphatases, total and available phosphorus. For total and available phosphorus (TP and AP), the soil was collected with a hand auger to a depth of 15 cm from 20 points in paper bags, thoroughly homogenized and transported to the laboratory. Samples for TP and AP were collected from each treatment at two different times: the first was between the two PSB inoculations, when the plants were at stem elongation, and the second at harvest. For the analysis of the two phosphatases, soil samples were collected from the 0-25 cm soil layer at harvest from five points on each treatment plot.

The soil was dried at room temperature and plant residues were removed from the samples. After that soil was sieved at a 2 mm diameter and stored in a dry place until analysis. For all analyses, samples were processed in triplicate. TP and AP were assessed by the molybdenum blue colorimetric method (Rusu et al., 2025) and the results were expressed as % for TP and ppm for AP. Acid and alkaline phosphatase activities were measured by incubating 1 g of soil at 37°C for 1 h. Spectrophotometric quantification was performed at an optical density of 420 nm. Results were calculated from the calibration curve equations and expressed as µg *p*-nitrophenol g^{-1} h^{-1} (Tabatabai & Bremner, 1969; Eivazi & Tabatabai, 1977, Aruna Kumari et al., 2018).

Statistical Analysis

SPSS Statistics 20 software was used for one-way analysis of variance (ANOVA) and comparing the means of fertilizer treatments both within and between tillage systems. This was followed by Tukey post-hoc test to determine differences between group means. Spearman rank correlation (2-tailed) was used to check the significance and direction of correlation of the studied indicators. This was performed for each tillage system individually.

RESULTS AND DISCUSSIONS

Acid and Alkaline Phosphatases

Acid and alkaline phosphatases are indicators of enzymatic activity and overall soil fertility, being involved in the hydrolysis of substrate phosphomonoesters and the release of available P (Banerjee et al., 2012; Futa et al., 2021). The use of fertilizers containing PSB contributes to increased phosphomonoesterases activity and provides accessible P through solubilization of mineral fertilizers (Chesti et al., 2013) or by decomposition of organic matter (Tahir et al., 2018).

Under our experimental field test conditions, the intensity of activity of both enzymes varied with fertilizer treatments and tillage.

The acid phosphatase activity ranged from 1.16 µg *p*-nitrophenol g^{-1} h^{-1} in T3 from CT soil to 1.86 µg *p*-nitrophenol g^{-1} h^{-1} in RT control (T0) (Figure 2).

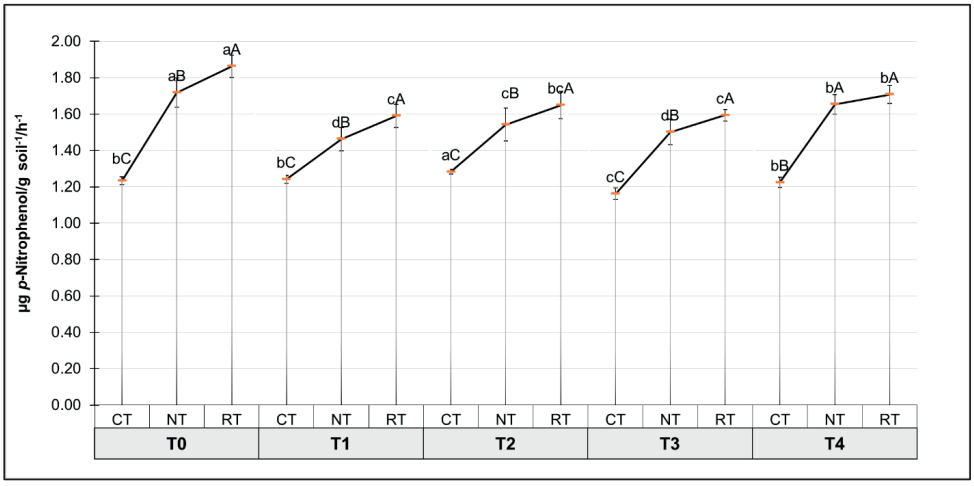


Figure 2. Acid phosphatase activity

Note: Distinct lower case letters express that the differences are significant between fertilization treatments within the same tillage system; distinct upper case letters express that differences are significant for the same fertilization treatment within different tillage systems

Of the tillage practices, in CT within fertilizer treatments, inoculation of 75% PSB + N (T2) was significantly higher (1.28 µg *p*-nitrophenol $g^{-1}h^{-1}$) than all other treatments. At the opposite extreme, T3 was found to produce the lowest acid phosphatase activity. Between T0, T1 and T4, there were no significant differences and the means were 1.23, 1.24 and 1.22 µg *p*-nitrophenol g^{-1} h^{-1}, respectively.

Results were similar in NT and RT soils, with T0 significantly above bacterial fertilizations (1.72 µg *p*-nitrophenol g^{-1} h^{-1} in NT and 1.86 µg *p*-nitrophenol g^{-1} h^{-1} in RT).

Between tillage systems, acid phosphatase activity was significantly greater in RT, followed by NT and then CT, a tendency that lasted regardless of fertilization treatment.

Alkaline phosphatase originates mainly from bacterial synthesis. Inoculation at the recommended rate of *Bacillus megaterium* var. *phosphaticum* without N supply in the CT system promotes alkaline phosphatase synthesis (0.28 µg *p*-nitrophenol g^{-1} h^{-1}) significantly higher than control and the other bacterial treatments (Figure 3). No significant changes in alkaline phosphatase activity were observed between T0, T3 and T4, whereas the mean activity of T2 was significantly reduced.

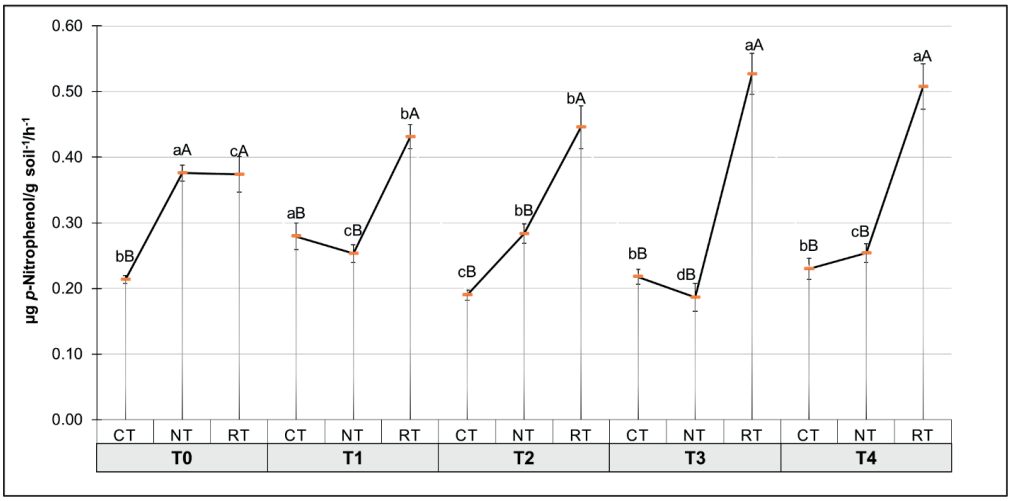


Figure 3. Alkaline phosphatase activity

Note: Distinct lower case letters express that the differences are significant between fertilization treatments within the same tillage system; distinct upper case letters express that differences are significant for the same fertilization treatment within different tillage systems

An opposite trend was found in the NT system, where the mean value of the control (0.38 µg *p*-nitrophenol g^{-1} h^{-1}) was significantly higher than that of bacterial treatments. Of the bacterial inoculations, T2 ranked first, close but with a significant decrease, followed by T1 and T4. The lowest activity was measured in T3 soil samples (0.19 µg *p*-nitrophenol g^{-1} h^{-1}).

Alkaline phosphatase activity increased with microbial inoculation under RT with all treatments significantly above control.

The general trend of alkaline phosphatase activity measurements in all three soil management practices was the same as for acid phosphatase (RT > NT > CT). However, differences between CT and NT were significant only in the control, and for treatments T1 and T3, mean values in the CT system slightly exceeded those in NT.

Total and Available Phosphorus

Phosphorus (P) is a nutrient with multiple constitutive and functional implications in plants. The amount and availability of soil P depends on soil type, SOM, biological activity, pH, climatic conditions and land management in terms of cultivated crops, soil tillage and fertilization.

In our study, analysis of soil TP content showed mean values between 0.0191 and 0.0542% and significant differences ($p < 0.05$) across both fertilization treatments and tillage practices (Figure 4).

In CT system, soil TP levels remained on the same tendency at both times of the determinations. The control outperformed the *Bacillus* sp. treatments, with means of 0.0410 - 0.0450% and T2 (75% PSB + N) reported significant lowest values (0.0343 - 0.0307%). Of the bacterial treatments, T1 and T4 were similar in total P content, significantly higher than T2 and T3. There were no significant differences between control, T1 and T4 in the samples collected at crop harvest.

Instead, in the NT system, between the two bacterial inoculations, a significantly higher

level of TP was observed in T3 (0.0433%), and between the other treatments the variation in means was slight and insignificant. At harvest, in NT the total P level fluctuated quite widely, and the ascending order of averages was T1 > T0 > T4 > T3 > T2.

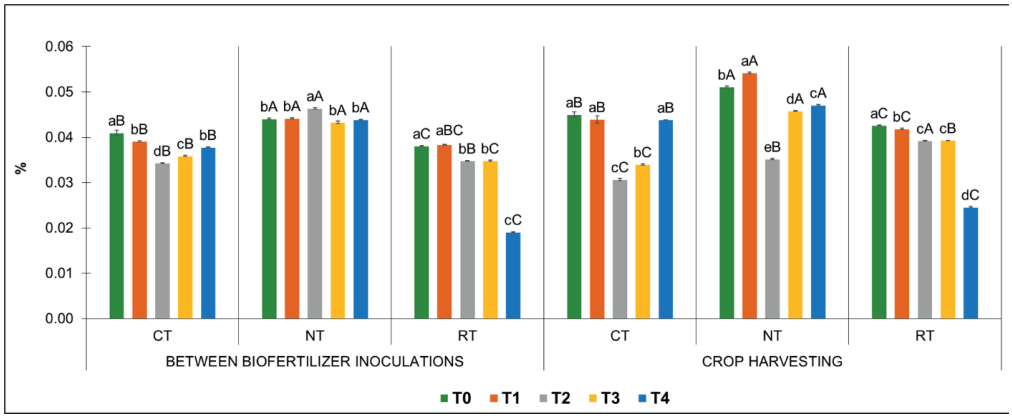


Figure 4. Soil total phosphorus content

Note: Distinct lower case letters express that the differences are significant between fertilization treatments within the same tillage system; distinct upper case letters express that differences are significant for the same fertilization treatment within different tillage systems

In the RT system, the same behavior of the fertilizer treatments was maintained at both soil sampling times. The highest means were recorded in T0 and T1 (100% PSB), followed by T2 and T3 and finally T4, with significantly lower values. As a general pattern among bacterial treatments, 100 % inoculation without the addition of N fertilizer resulted in higher total soil phosphorus content.

Comparing the tillage systems with each other, for the same fertilization treatment, between the two inoculations with *Bacillus megaterium* var. *phosphaticum*, NT ranked first in terms of TP level, with significantly higher averages, followed by CT and RT. Also, at crop harvest the mean values were highest in NT, except for T3.

Even if soil TP levels are constant and substantial, the accessibility of this element to crops requires careful assessment to ensure optimal plant nutrition. Figure 5 shows soil AP levels at the two sampling times, highlighting the differences between fertilization treatments as well as between tillage systems.

Comparatively, the mean values in CT, NT and RT, reveal a decrease in AP from the time of the first soil sampling until crop harvest. There were also significant differences between fertilizer treatments in all three systems.

In the first analyses, in CT and RT systems the highest averages were found in T4 (38.48 and 38.32 ppm) and under NT in T3 (50.23 ppm). In both CT and RT, the control (T0) ranked second with an AP level of 31.49 and 44.34 ppm, and the lowest values were found in T2 (22.80-34.08 ppm). In RT system all PSB variants outperformed the control. At wheat harvest, the evaluation of the soil accessible phosphorus content in the CT system resulted the maximum value in the control (27.20 ppm), significantly exceeding the other treatments. In contrast, in NT, biological inoculations had the highest values (T3 > T4 > T1 > T2). Finally, in the RT system, the highest mean was obtained in T4 (37.31 ppm), followed by the control (31.34 ppm), T3 (25.92 ppm), T2 (21.755 ppm) and T1 (20.10 ppm).

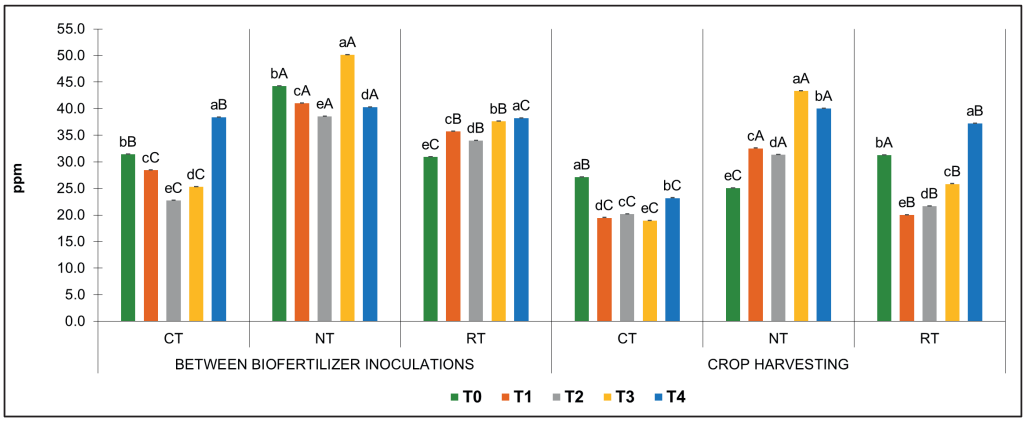


Figure 5. Soil available phosphorus content

Note: Distinct lower case letters express that the differences are significant between fertilization treatments within the same tillage system; distinct upper case letters express that differences are significant for the same fertilization treatment within different tillage systems

The lower accessibility of P could be observed in the conventional system compared to NT and RT.

Spearman correlation revealed the significance of the relationships between the investigated properties. (Table 4).

Table 4. The correlation between the indicators considered in the study for each tillage system

Tillage system	*Parameter*	**Acid phosphatase**	**Alkaline phosphatase**	**TP**	**AP**
CT	**Acid phosphatase**	1			
	Alkaline phosphatase	0.156	1		
	TP	-0.042	0.227	1	
	AP	0.570*	0.402	0.511	1
NT	**Acid phosphatase**	1			
	Alkaline phosphatase	0.560**	1		
	TP	0.046	-0.157	1	
	AP	-0.868**	-0.218	-0.189	1
RT	**Acid phosphatase**	1			
	Alkaline phosphatase	0.469**	1		
	TP	0.234	0.316	1	
	AP	0.396	0.634*	-0.322	1

Note: * means that correlation is significant at the 0.05 level (2-tailed); ** means that correlation is significant at the 0.01 level (2-tailed).

The analysis indicated a significant and moderate positive correlation in NT and RT systems between acid and alkaline phosphatase activities (rs = 0.560, p = 0.026 in NT and rs = 0.469, p = 0.00002 in RT). AP was found to have a positive and moderate correlation with acid phosphatase under CT system (rs = 0.570, p = 0.026), but negative and very strong in NT (rs = -0.868, p = 0.00003). In contrast, in RT, AP was positively and strongly correlated (rs = 0.634, p = 0.011) with alkaline phosphatase.

Acid phosphatase is of microbial and roots origin (Eichler et al., 2004) and its activity is modulated by air and water regime, soil management, SOM content, soil chemistry, plant species and their physiological state (Ciereszko et al., 2011; Adetunji et al., 2017). The higher values of acid phosphatase compared to alkaline phosphatase can be attributed to the low acid pH of the soil.

The external addition of *Bacillus megaterium* var. *phosphaticum*, together with mineral fertilization, showed an increase in acid phosphatase activity, both as a function of inoculated dose and the type of soil management. The increase in acid phosphatase activity induced by inoculation of wheat with P solubilizing bacteria consortium (*Bacillus siamensis*, *Rahnella aceris*, *Bacillus cereus*) was also reported by Ibnyasser et al. (2024).

In soil inoculated with PSB, we found that both lower (75 %) and higher (125 %) doses, together with N addition, increased the synthesis of this

enzyme. Microbial enrichment promoted enzymatic activity to catalyze reactions to convert P from inaccessible forms into plant-available P from organic compounds or mineral fertilizers. Mineral fertilization, in particular with N, leads to enhanced acid phosphatase synthesis due to its acid pH-related activity (Lemanowicz, 2011; Kalembasa & Symanowicz, 2012). However, in our study, in the NT and RT systems, in treatments with the same *Bacillus* rate (100%) but different N supply (T1 and T3), this nutrient had no obvious effect on acid phosphatase activity. In CT, even a significant inhibition of this enzyme was observed with the addition of N. This is in opposition to the results obtained by Widdig et al. (2019), who found that N fertilization leads to increased phosphatase activity. We can assume that the differences between biofertilizer treatments mean (T1, T2, T3 and T4) come from a combined effect of PSB inoculum dose and nitrogen. When inoculating soil with acid-lactic acid bacteria, *Pseudomonas* spp., *Penicilium*, *Actinomycetes* spp., Długosz and Piotrowska-Długosz (2023) identified an inhibition of phosphomononosesterase activity, as we reported in NT and RT systems, but an increase in available P content. Thus, acid phosphatase activity decreased from 2.20 in the control to 2.16 mM *p*NP kg^{-1} h^{-1} in the inoculated treatment, alkaline phosphatase from 2.17 to 1.68 mM *p*NP kg^{-1} h^{-1}, and AP increased in the biological inoculum treatment to 79.4 from 66.6 mg kg^{-1} in the control (Długosz and Piotrowska-Długosz, 2023. Also, Sednev (2017) found no influence of *Bacillus megaterium* var. *phosphaticum* inoculation with N on phosphatase activity or P content and a low and insignificant positive correlation between soil P level and phosphatase activity. Biological inoculum consisting of several *Bacillus* species and active organic matter in combination with $NPK_{20:10:10}$ application was shown to stimulate alkaline phosphatase. In addition, it was suggested that mineral fertilizers can be replaced by biofertilizers to increase enzyme activity and thus more efficiently utilize soil P pools by the wheat crop (Ali et al., 2024).

A characteristic feature of the investigated enzymes is the higher activity in the RT system compared to CT and NT, and these differences are related to the variation in soil properties. Soil disturbance in RT after a long period of no-tillage resulted in a balanced moisture and aeration regime and, as a result, stimulated microbial activity, with positive effects on enzymatic activities across treatments. Also, in the NT system, acid phosphatase increased significantly compared to CT, but alkaline phosphatase was in all cases insignificant, although higher in T2 and T3, confirming our hypothesis. Soil phosphatase activity was found to be sensitive to soil management (Gałązka et al., 2017). Soil microbial community has a key role in available P supply (Rodríguez and Fraga, 1999), which means that reduced disturbance induces increased enzymatic activity. Due to the deposition of plant residues in the surface layers corresponding to the soil sampling depth, acid phosphatase and alkaline phosphatase are abundant in soils cultivated in NT and RT (Mohammadi, 2011), a large part of soil P being organic (Nannipieri et al., 2011). Minimal soil disturbance associated with mulching creates a suitable environment for the interaction between soil physical-chemical parameters and the functioning and proliferation of microorganisms, ultimately facilitating biochemical P conversion mediated by soil enzyme systems (Calistru et al., 2024). The higher enzymatic activity in NT and RT systems can also be explained by the optimal temperature and humidity provided by the mulch layer, which prevents sudden temperature changes and rapid evaporation of water, with a positive effect on microbial activity (Mathew et al., 2012). The two enzymes studied were found to have a positive feedback interaction, as indicated by Spearman`s correlation, consistent with the research of Samuel et al. (2021).

Several bacterial strains selected from the rhizosphere have revealed the potential to enhance P solubilization and efficient uptake, contributing to an environmentally friendly approach to plant nutrition (Selvi et al., 2017; Emami et al., 2020). Many studies report the viability of inoculating PSB in converting P from soil-fixed P pools or from crop fertilization, with growth and yield promoting effects. The higher level of accessible P in the treatments with 125% + N in CT systems and 100% PSB + N in NT systems suggests that the metabolic mechanisms of the microorganisms were effective in solubilizing a fraction of the

soil P reservoir and aligns with our hypothesis. Chen et al. (2021) found that PSB (*Enterobacter hormaechei*, *Pseudomonas grimontii*, *Pantoea roadsii*) can contribute to the increase in total and accessible P content as well as acid phosphatase activity intensity. However, in our study, we generally recorded a decrease in soil TP in bacterial inoculum treatments. Inoculation with PSB has been reported to significantly increase soil P availability and phosphatase activity in wheat crop and a positive correlation between them (Yahya et al., 2021). In our study, there was a positive effect of PA on acid phosphatase in the CT system and on alkaline phosphatase in the RT system, but an inhibitory effect on acid phosphatase in the NT. Increased secretion of phosphatases by soil microbiota and plant roots is related to a lack of available soil P (Kai et al., 2002; Dick et al., 2011).

When comparing tillage systems in terms of soil P content, the NT was found to have the highest level, followed by CT and then RT. This finding is in contradiction with that of Futa et al. (2021), who reported higher available P content in wheat crop at deep mineral fertilizers application. One explanation for this phenomenon observed in our study may be that, in minimum tillage systems, nutrients accumulate in the surface soil layers (Mühlbachová et al., 2024). Accessible P decreased from the first measurements stage until the end of the growing season, which means that a part of it was taken up by the root system and metabolized in plants, and some was converted into inaccessible forms.

CONCLUSIONS

The success of soil colonization by inoculated bacteria depends on the interaction of several factors. Thus, native microorganisms, soil properties, crop species and climatic conditions all have the potential to interfere with this process, preventing the bacteria from reaching their growth and development potential (Chen et al., 2021). Inoculation of *Bacillus megaterium* var. *phosphaticum* promoted phospho-monoesterase activity in NT and RT soil management systems. Application of recommended or higher rates (100% or 125%), along with N, also increased available soil P in all tillage systems. Minimal soil disturbance provides a favorable environment for microbial activity, stimulation of enzyme activity and P accumulation, with positive effects on plant nutrition. The mechanisms of action of BMPs are diverse and the direct or indirect influences they may have on soil P require further investigation and other research directions.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

INCREASED ACTIVITY OF SPECIALIZED FUNCTIONAL MICROBIOMES IN HISTORICALLY POLLUTED SOILS AFTER BIOREMEDIATION

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Abstract

Soil heavy metal pollution is one of the major concerns in urban habitats, due to the associated human and environmental risks. Materials and methods: The aim of this research is to use the DEMSA model to explore the increases in the soil microbial functional profile, based on Biolog Ecoplates results, due to historical pollution with heavy metals. The historical pollution of the 5 sites analyzed induces a high variability in the soil functional microbiomes. The functional intensification is visible in the sum of all activities and the Index of Intensification from each site. At this niche level the Carboxylic and acetic acids guild shows the highest share of activities. Above the intensification level, a guild expansion is observed only in three of the analyzed sites. For these sites, the Index of Expansion is set in the interval of 2.5-6.0, which indicates the presence of multiple functional groups adapted to heavy metals. Historical pollution alters the assemblage of soil general microbial community, by increasing the activity of a small number of very specialized groups.

Key words*: functional increase, intensification matrix, guild expansion, index of alteration.*

INTRODUCTION

Soil pollution with heavy metals in urban areas present an important concern and a high problem for both human and ecosystem health (Binner et al., 2023). In multiple urban areas, historical anthropic activities led to increased levels of heavy metals which had been accumulated in soils and represent a serious threat to human health (Adewumi & Ogundele, 2024). Heavy metals produce a strong destabilizing effect on soil microbial communities, altering their temporal stability and functional profiles, which affect their ecosystem services (Tang et al., 2024). Thus, a more sensitive approach in monitoring and understanding the risks related to heavy metal levels from soils is important in the selection of most suitable methods to remove them from ecosystems (Ahirvar et al., 2023; Shi et al., 2023).

Bioremediation is a viable solution for removing heavy metals from environment, and rebuild plant and microbial communities (Pande et al., 2022; Verma et al., 2021). This process sustains the recovery of soil microbiome and enhance the ecological restoration (Zheng et al., 2024). Constant monitoring of bioremediation is necessary to understand the efficiency of the process, and to forecast vegetation and soil changes (Kuppan et al., 2024; Sales et al., 2020; Bala et al., 2022). These approaches are necessary to constantly increase the heavy metal removal by integrating supplementary solutions (Azhar et al., 2022; Liu et al., 2020; Wang et al., 2022).

Microbial communities are defined by complex assemblages and interactions, based on high species diversity and functions. Based on these traits, the assessment of soil microbiomes and the changes in their activity and community profile, represent a suitable method for the detection of heavy metals impact (Abbaszade et al., 2023; Melloni et al., 2023; Tang et al., 2024).

The aim of this study is to analyze the increasing activities trends in microbial functional profile from 5 historically contaminated soils with heavy metals, after one year of phytoremediation. The proposed objectives are: i) the assessment of microbial functional profile toward an urban standard; ii)

the analysis of functional guilds that exceed the activity in urban standard; iii) the assessment of functional alteration and diversity changes in microbial profile.

MATERIALS AND METHODS

Soils from 5 historically polluted sites in Baia Mare (Baia Mare County) were sampled after one year of phytoremediation (Pop et al., 2024). The five sites selected for analysis were: Craica (CR), Colonia Topitorilor (CT), Ferneziu (FR), Urbis (URB), Romplumb (ROMP).

Samples were diluted to 10^{-4} and were subjected to inoculation in Biolog Ecoplates, for each sample being selected 3 replications. The entire incubation last 96 hours, with reading performed at 590 nm every 24 h for the detection of changes in microbial activity.

Values of all readings at 96 h, separately for each substrate, were used to calculate the average standard of microbial functional activity. To this average, the +/- standard deviation was applied to set the minimum and maximum potential activity. These three values for each substrate were used as a control in the Detailed Exploration of Microbial Sociological Assemblage (DEMSA) model (Stoian et al., 2022). Based on comparison with the control, all the values that exceeded the average, but were below the maximum were used to build the intensification matrix. All the data that exceeded the maximum of the control were assembled in the expansion matrix. Following Stoian et al. (2022) method, each substrate was considered as a functional group, and each functional groups was associated with a functional guild, based on substrate chemical traits. The functional guilds used in the assessment of pollution impact were Carbohydrates (CH), Polymers (P), Carboxylic & acetic acids (CX), Amino acids (AA), Amines/amides (AM).

The procedure was applied to score the most reduced changes in each of the site microbial functional profile, compared to the general microbial activity within the urban area. All data were analyzed in RStudio (R Core Team, 2024), with formulas from packages “psych” (Revelle, 2024) - basic statistics, “agricolae” (de Mendiburu, 2023) - ANOVA and LSD test, and “vegan” (Oksanen et al., 2022) - NMDS ordinations. All functional guilds were analysed for their potential interaction with correlations form the package “Hmisc” (Harrell, 2025).

RESULTS AND DISCUSSIONS

The use of the DEMSA concept in screening the changes of soil functional microbiome from polluted soils provides a detailed perspective on both the diversity of activities and community groups (Stoian et al., 2022; Vidican et al., 2016). Intensification of activities is analyzed as the proliferation of guilds (and groups) within the upper limits of the standard community. In comparison, expansion is analyzed as activities that exceed the maximum limit of the standard community and present a specialized proliferation in functional community.

Microbial functional guilds indicate an intensification of activities and present a large variation between sites (Table 1). The mean of activities extracted in the Intensification matrix showed the greatest values associated with CH and CX guilds. CT site presented a maximum of activity, but the differences compared to next two sites (CR and FR) was not significant. An interesting phenomenon was observed for sites ROMP and URB, where the activity was 20 to 200 times lower. This difference indicates a significant decrease in the microbial population in this guild. In terms of CX activity, the greatest values were observed in FR site, with a similar difference compared to ROMP and URB.

Between FR and CR, a significant difference was detected, which offer a gradient of activities in this functional guilds (Table 1). The gradient show two sites that present a higher activity in FR and CT, a medium activity in CR and very low activities in ROMP and URB. For the P, AA and AM guilds ROMP present no activity, while URB microbiome showed reduced activities. Both P and AA presented similar activities associated with FR conditions, while AM reduced its activity at half. AA presented higher activities in CR and FR compared to P and AM guilds, which indicate a better condition for this type of microbiome.

Based on functional guilds, the sum of recorded activities presented large variations between sites (Table 1). CT sites showed the highest sum of activities, with two absorbance units higher than in FR site. CR site present a 6 absorbance unit reduction of microbial activity, while for ROMP and URB this parameter was under 1 unit. These values are visible in the values of Intensification Index, which scores the site-specific changes in the entire microbiome that exceed the average limits of the activity standard.

Table 1. Microbial functional guilds and diversity in Intensification range of activities

Sites	CH	P	CX	AA	AM
CR	2.27±1.17a	0.56±0.14c	2.75±0.24b	1.67±0.22b	0.18±0.18b
CT	4.03±0.16a	2.06±0.16a	3.79±0.62a	2.77±0.50a	1.11±0.03a
FR	2.46±0.46a	1.29±0.12b	4.37±0.07a	2.66±0.46ab	0.84±0.11a
ROMP	0.02±0.02b		0.05±0.05c		
URB	0.23±0.23b	0.09±0.09d	0.24±0.24c	0.05±0.05c	0±0b
Sites	Sum	Index of	Shannon	Simpson	Pielou
CR	7.45±1.02b	17.9±1.56b	2.56±0.15a	0.91±0.01ab	0.94±0.01a
CT	13.7±0.85a	25.3±1.10a	3.31±0.02a	0.96±0.00a	0.97±0.00a
FR	11.6±0.54a	22.6±0.82a	3.06±0.02a	0.94±0.00a	0.93±0.01a
ROMP	0.16±0.13c	0.76±0.55c	0.25±0.25b	0.49±0.28b	0.23±0.23b
URB	0.62±0.62c	2.05±2.05c	0.55±0.55b	0.92±0.07ab	0.26±0.26b

Note: values ± s.e. followed by different letters present significant differences according to LSD ($p<0.05$). Legend: sites - Craica (CR), Colonia Topitorilor (CT), Ferneziu (FR), Urbis (URB), Romplumb (ROMP); guilds - Carbohydrates (CH), Polymers (P), Carboxylic & acetic acids (CX), Amino acids (AA), Amines/amides (AM); Index of - Index of Intensification.

The highest change in the microbiome pattern was near to 25% in CT site (Table 1). This value indicates a shift in over a quarter of functional microbiome, followed by FR site where this index is set to 22%. At opposite, ROMP maintained its functional community near to the standard, which indicate that soil conditions significantly restrict the entire microbial community.

Diversity indices showed different values that enable the qualitative scoring of the functional microbiome assemblage (Table 1). Shannon-Wiener diversity index (Baliton et al., 2020) showed a diverse community in both CT and FR sites, that indicates a microbial community adapted to the site conditions.

A moderate diversity is visible in CR site, while both ROMP and URB showed very low diversity. Based on this index the sites with low values presented only a fraction of their microbial community that can exceed the area standard, which can be visible in a longer period for the restoration of an intense activity. For the sites with high diversity, the number of functional groups in community is higher and is associated with a coupled activity that can progress faster toward a restoration point.

The Simpson diversity index was used to score both the number of functional microbiomes in each site and their distribution within community (Johnson & Burnet, 2016; Vidican et al., 2016). For ROMP site, the functional microbiome presented a medium heterogeneity, while for the rest of 4 sites this index showed a high heterogeneity (Table 1). This implies a specific localized reaction of soil microbiome that is not replicable in other sites, even in the same area.

There is a large uniformity of functional microbiomes in CT site, and an almost balanced community for CR and FR sites, based on Pielou index. On the other way, the values of this index in ROMP and URB showed unbalanced evenness in community.

The analysis of functional microbiome that exceed the maximum activity of the control was assembled in the Expansion matrix (Table 2). Compared to the Intensification segment of activities, for this community is not present in ROMP and URB sites. All sites presented lower activities that suggest a functional microbiome that is able to proliferate in pollution conditions, but not completely adapted to these. CT site presented the highest activities for CH, P and AA functional guilds, while CX and AM were associated with FR site, both communities presenting a different pattern of consortium. The most visible differences were observed in CX guild, with a significant decrease from FR to CR site. The reduction coefficient for this guild is 2-2.5 times between sites. The sum of activities showed significant differences between CT, FR and CT sites. For the last site, the sum of all activities does not exceed the activity of CH guild in CT site.

In terms of functional modification, the microbiome that exceeds the limits of the standard ecological niche present values in a range between 2.5-6.0 (Table 2). This implies a reduced change in the community assemblage pattern, with the only a reduced potential for evolving toward a highly different pattern.

Diversity analyzed through all three indices shows a moderate community associated with CT conditions, while for FR the value is at the limit between low and moderate (Table 2). CT

site presented a very low diversity, which implies a specialized community that can proliferate in this conditions. The higher values for the other two sites indicates a larger functional microbiome that can exceed the standard limits and proliferate in pollution conditions. In terms of pollution effect, Shannon index present a medium to low effect on functional communities. The Simpson index show a medium to high heterogeneity of microbiome, which indicate a future potential development of multiple specialized groups that can use at a higher level soil conditions. Pielou`s index show an almost balanced community, with functional activities that maintain a similar pattern.

Table 2. Microbial functional guilds and diversity in Expansion range of activities

Sites	CH	P	CX	AA	AM
CR	0.33±0.28b		0.20±0.10c	0.44±0.24ab	0.07±0.07ab
CT	1.10±0.25a	0.27±0.18	0.62±0.12b	0.99±0.19a	0.21±0.12ab
FR	0.45±0.28ab	0.08±0.04	1.39±0.19a	0.52±0.29ab	0.26±0.09a
Sites	Sum	Index of	Shannon	Simpson	Pielou
CR	1.06±0.11b	2.56±0.14b	1.19±0.28b	0.64±0.09b	0.90±0.03a
CT	3.21±0.35a	5.90±0.64a	2.28±0.03a	0.87±0.01a	0.90±0.03a
FR	2.73±0.16a	5.31±0.26a	2.00±0.14a	0.83±0.02ab	0.88±0.02a

Note: values ± s.e. followed by different letters present significant differences according to LSD ($p<0.05$). Legend: sites - Craica (CR), Colonia Topitorilor (CT), Ferneziu (FR); guilds - Carbohydrates (CH), Polymers (P), Carboxylic & acetic acids (CX), Amino acids (AA), Amines/amides (AM); Index of - Index of Expansion.

The differences between sites in terms of intensification potential is visible in the position on NMDS ordination graph (Figure 1). From all 5 sites, ROMP and URB show the highest stability, with points placed in the same proximity. The CT, CT and FR sites showed a higher difference in the population of their specific communities. The position of CR, ROMP and URB on the opposite side of guild vectors implies a combined effect of multiple functional groups for shaping the assemblage of intensified community. The rest of the two sites showed a position in the minus quadrats defined by axis 1.

Both P and AM guild vectors are oriented toward the centroid of CT group, the vectors indicating these two guilds as potentially responsible for the future changes within the community (Figure 1). The two guilds have a medium position in the community, which makes them good candidates for reorganizing the entire community by an increase in their activity.

An interesting case was observed based on the position of FR plots on ordination (Figure 1). One functional community presents a similar change pattern with the centroid of CT sites. The position near to P vector indicates for this point a higher importance of P guild in the transformation of functional community. The rest of the two plots showed divergent directions of evolution. One plot is oriented toward CR community, outside the influence of a specific guild. For this site there is a potential change due to multiple group interaction. The third plot is placed on the SUM vector direction, which indicates an increase in the global functional activity.

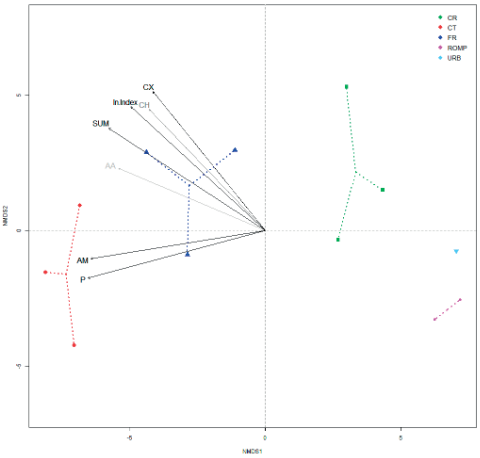


Figure 1. NMDS projection of microbial communities in Intensified niche

Legend: sites - Craica (CR), Colonia Topitorilor (CT), Ferneziu (FR), Urbis (URB), Romplumb (ROMP); In - Index of intensification; H - Shannon index; S - Simpson index; guilds - Carbohydrates (CH), Polymers (P), Carboxylic & acetic acids (CX), Amino acids (AA), Amines/amides (AM)

The Index of intensification vector is placed near to this site and indicates a highly potential change within the community that will evolve into a different pattern of microbiota (Figure 1). AA and CH vectors have a lower impact on the development of functional community. Their position indicates an additional effect of the two guilds for the future shape of microbial assemblage.

The projection of sites that showed an expansion potential in the functional microbiome indicates three different microbial community structure (Figure 2). The most stable and similar community is observed in

CR site, with points placed near to the center of the ordination and with a reduced space between them. The second site in terms of similarity and stability of community is FR, with a point in the ++ quadrat and two points in the +– quadrat. The first point shows a direction of evolution toward CX and AM vectors, indicated the impact of these two guilds in the future development of the community. The last two points showed a divergent orientation and no correlation with any of the guild vectors. This indicates a different pattern of functional activities and a microbiome that will evolve due to the interactions between multiple functional groups.

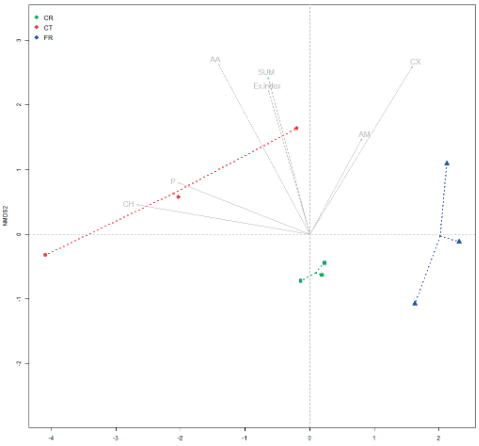


Figure 2. NMDS projection of microbial communities in Expansion niche

Legend: sites - Craica (CR), Colonia Topitorilor (CT), Ferneziu (FR); In - Index of Expansion; H - Shannon index; S - Simpson index; guilds - Carbohydrates (CH), Polymers (P), Carboxylic & acetic acids (CX), Amino acids (AA), Amines/amides (AM)

The CT points show an interesting projection on the ordination. Two points are placed at a large distance, while the third point is positioned in the middle of this distance (Figure 2). This last point has a microbiome associated with the P and CH guild activity, that are responsible for the development of the community. The most interesting case is represented by the point from CT site that is placed in the upper part of the ordination. Its position is near the vectors represented by the sum of activity and the index of expansion, both suggesting an alteration of the normal shape of community due to an increase in the sum of activities. This point is also placed between AA and CX(+AA) vectors, even at a larger distance, which indicate the role of these guilds in the assemblage of future and reorganized community.

At guild level, groups showed a significantly different importance in the projection of their communities on NMDS ordination (Table 3). The highest significant in the shape of intensification communities is associated with CX2 and AA6 functional groups. Compared to these groups, numerous ones showed a distinct importance for the development of soil functional microbiomes. Each functional guild present two distinct groups, except AM guild - where each functional group have a different level of impact. Twelve functional groups presented a significant impact for the development of their communities, most of them in the carbohydrates guild.

Table 3. Microbial functional groups associated with Intensification and Expansion in the range of activities

Niche segment	Group	Sig	Group	Sig	Group	Sig
Intensification	P1	*	CH3	*	CX1	*
	P3	**	CH4	*	CX2	***
	P4	**	CH5	*	CX3	**
	AA2	*	CH6	*	CX4	*
	AA3	*	CH8	**	CX7	*
	AA4	**	CH9	**	CX8	**
	AA5	**	CH10	*	AM1	**
	AA6	***			AM2	*
Expansion	AA3	**	CH3	*	CX4	*
	AA4	**	CH6	*	CX8	*
	AA5	*				
	AA6	*				

Note: groups assessed with * showed a significant impact in NMDS ordination (*p<0.05*; p<0.01**; p<0.001* ***). Legend: functional groups – P1 – Tween 40, P3 – α-Cyclodextrin, P4 – Glycogen, CH3 – α-d-Lactose, CH4 – β-Methyl-d-glucoside, CH5 – d-Xylose, CH6 – i-Erythritol, CH8 – N-Acetyl-d-glucosamine, CH9 – Glucose-1-phosphate, CH10 – d,l-α-Glycerol phosphate, CX1 – d-Glucosaminic acid, CX2 – d-Galactonic acid γ-lactone, CX3 – d-Galacturonic acid, CX4 – 2-Hydroxy benzoic acid, CX7 – Itaconic acid, CX8 – α-Keto butyric acid, AA2 – l-Asparagine, AA3 – l-Phenylalanine, AA4 – l-Serine, AA5 – l-Threonine, AA6 – Glycyl-l-glutamic acid, AM1 – Phenylethylamine, AM2 – Putrescine.

In terms of expansion, only a reduced number of functional groups can act significantly in the development of new community groups. From these, two functional groups show significant importance for each of CH and CX guilds (Table 3). An interesting case was observed for AA guilds, where 4 functional groups showed different levels of significance. Both P and AM associated groups have a reduced singular impact in the development of soil microbiome.

At community level the interactions between functional guilds are responsible for the development of a specific assemblage and diversity (Table 4). All the correlations between parameters and indices in the intensification niche are significant ($p<0.05$). This indicate a coupled activity and positive influence between functional guilds. This type of interaction is characteristic to polluted soils where the number of microorganism is reduced and their potential niche is not completely overlapped. Also, this type of community present functional microbiomes that are more generalist that specialized and the community maintain its diversity. All the functional guilds are responsible for the growth of the total activity, and between them are visible positive interactions. The highest level of association between two guilds is 0.90 (AA and CX), a value that indicate a coupled activity. CX and AA guilds are responsible for the highest alteration of microbial assemblages, their activities favoring a shift in the community. An important value is the 0.99 correlation between the sum of activity and the index of alteration, which sustain the constant change of microbial community shape as the sum of activities increase. These two parameters are responsible for the increase of diversity (H) and the development of a heterogenous microbiome (S). Heterogeneity is achieved in the community based on the Shannon index increase along with the alteration observed in the microbiota. Both CX and AA guilds are responsible for the sustain of heterogeneous cluster, and lead to an increase in the Simpson index as their activity increase.

Table 4. Interrelations between microbial guilds and indices in Intensification and Expansion niche

									Intensification
	P	CH	CX	AA	AM	Sum	In	H	S
P		*0.77*	*0.80*	*0.85*	*0.88*	*0.90*	*0.87*	*0.84*	*0.70*
CH	0.49		*0.81*	*0.73*	*0.71*	*0.90*	*0.89*	*0.86*	*0.72*
CX	0.16	0.37		*0.90*	*0.80*	*0.96*	*0.96*	*0.94*	*0.82*
AA	0.37	0.48	0.48		*0.79*	*0.93*	*0.93*	*0.89*	*0.76*
AM	0.11	0.40	*0.66*	0.34		*0.87*	*0.81*	*0.78*	*0.63*
Sum	0.50	*0.78*	*0.79*	*0.79*	*0.65*		*0.99*	*0.95*	*0.81*
In	0.49	*0.76*	*0.79*	*0.79*	*0.64*	*1.00*		*0.98*	*0.87*
H	*0.53*	*0.78*	*0.76*	*0.65*	*0.68*	*0.95*	*0.96*		*0.94*
S	0.38	*0.59*	*0.65*	*0.69*	*0.59*	*0.82*	*0.87*	*0.90*	
Expansion									

Note: values in *italic* present significant correlations ($p<0.05$). Legend In - Index of alteration; H - Shannon index; S - Simpson index; guilds - Carbohydrates (CH), Polymers (P), Carboxylic & acetic acids (CX), Amino acids (AA), Amines/amides (AM).

In the Expansion niche (Table 4) the interrelations between guilds and parameters present lower values. There are no significant positive interrelations between functional guilds, this indicating a specialized niche and a complete different metabolism for each group. In the expansion niche are present the most adapted microorganism and their activity is oriented toward a separated area and resources. None of the functional guilds contribute to a powerful increase in the sum of activities or to a significant shift in the community groups. Both the sum of activities and the index of alteration are completely dependent. This indicates a restricted microbial community, that can proliferate in polluted soils and will evolve toward a more diverse population. These microorganisms can serve as a core for the development of a successional diverse community. Any activity recorded in functional guilds produce an increase in the diversity of the entire microbiome, leading to a more heterogeneous assemblage.

CONCLUSIONS

An increase in the activity, higher than 7.45 absorbance units, associated with the intensification segment of ecological niche produces a functional alteration of microbiomes in a range of 17.9-25.3%.

Activities in the microbial communities that exceed maximum levels are associated with low functional diversities and heterogeneous community assemblages.

Intensification is visible in multiple scenarios for the future development of communities and their functional assemblages, based on the specific reaction in most proliferative guilds.

Expansion in site Craica show a functional stability, while for Colonia Topitorilor was observed a divergent pattern of evolution.

A number of 23 functional groups can present intensified activities, while for the expansion profile only 8 of them present the ability of proliferation.

For both Intensification and Expansion segments of ecological niche an increase in the sum of activities leads to a proportional increase in the profile of functional communities and a constant reshaping phenomenon.

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CROP SCIENCES

Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

EFFECTS OF SOME BIOSTIMULATORS AT TWO POTATO CULTIVARS CULTIVATED AT DIFFERENT PLANT DENSITIES

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Abstract

The application of biostimulators to plants leads to the accumulation of a higher content of nutrients in their tissue and to positive metabolic changes improving plant nutrient use efficiency and tolerance to stress factors. The aim of this paper is to present the effects of some biostimulators at potato crop according to variety and plant density. The research was conducted in the years 2023 and 2024 at the National lnstitute of Research and Development for Potato and Sugar Beet Brasov, Romania. The experimental variants were the following: potato variety (Darilena; Ultra); plant density (45.000 plants/ha; 53.000 plants/ha); Biostimulator product (no biostimulator - control variant; Genaktis 1; Irys; Terram Number One; Wuxal Macromix). Compared to control (untreated) variant, all the used biostimulator products determined an increased number of stems per hill, an increased aerial part weight, and an increased tuber yield regardless the potato variety, plant density and climatic conditions of the year. The tuber yield increase determined by using biostimulators is higher in better climatic conditions and at higher plant density. Having all these into account, the inclusion of the biostimulators in the potato crop technology can be considered as a good strategy.

Key words*: biostimulator, potato, cultivar, plant density, plant traits.*

INTRODUCTION

Potato (*Solanum tuberosum* L.) is cultivated in about 160 countries all over the world and consumed every day by over a billion people (Camire et al., 2009; Bishwoyog and Swarnima, 2016). In the world production, potato is an important agricultural plant, not only on account of its consumer importance, but also due to its industrial and fodder significance (Zgórska and Grudzińska, 2012).

Plant biostimulator have been identified as one of the most important agronomic factors that scientists have been looking for in a range of natural sources over the last few decades (Yaseena and Takacs-Hajosa, 2022).

Biostimulator are substances applied to plants, intended to improve their status. Through the effects they produce, they are the equivalent of food supplements and vitamins for improving people's health. Thus, biostimulators help plants to grow and develop well and to go well through periods of stress.

In the actual context of climate change, biostimulators have gained increasing importance in supporting plants to overcome water stress.

Various biostimulators are classified based on application method (soil and foliar), source (plant and animal) or production method (hydrolysis, fermentation and extraction) (Suman et al., 2025). These natural products (seaweed extracts, humic substances, hydrolyzed proteins and products containing amino acids or microorganisms) contain a bioactive substance that improves plant growth, increases nutritional efficiency and tolerance to abiotic stress, improves plant protection against pathogens and improves plant traits and yield quality regardless of nutrient content (Calvo et al., 2014; du Jardin, 2015; van Oosten et al., 2017; Bulgari et al., 2019).

According to Regulation (EU) 2019/1009 of the European Parliament and of the Council, a plant biostimulator shall be an EU fertilising product the function of which is to stimulate plant nutrition processes independently of the product's nutrient content with the sole aim of improving one or more of the following characteristics of the plant or the plant

rhizosphere: nutrient use efficiency, tolerance to abiotic stress, quality traits, or availability of confined nutrients in the soil or rhizosphere.

The application of biostimulators to plants leads to the accumulation of a higher content of nutrients in their tissue and to positive metabolic changes.

For these reasons, the development of new biostimulators has become a point of scientific interest (Nardi et al., 2002). The biostimulator stand as powerful tools for more eco-friendly and productive agricultural systems (Suman et al., 2025).

The biostimulating effects of humic substances are characterized by both structural and physiological changes in plants, related to the absorption, assimilation and distribution of nutrients, as well as changes in the primary and secondary metabolism of plants related to tolerance to abiotic stress (Cioroianu et al., 2022).

The biostimulator action is to stimulate the development of leaves, stems and roots of plants, to supplement nutrient deficiency during the growing season caused, among others, by intensive plant development, drought or agrotechnical errors (Bienia et al., 2018).

Foliar treatments have been demonstrated to increase the efficiency with which macro- and micronutrients are absorbed and utilized (Karak et al., 2023).

An important element in potato agrotechnics is the "greening" process and the constant reduction of unit costs of production (Mystkowska, 2022). We are currently facing clearly visible climate changes and increasingly extreme weather conditions, which are accompanied by various stress factors for plants, like high and low temperatures, periodic droughts, floods, frosts (Abd EL-Wahab et al., 2016; Fleming et al., 2019).

In all traditional areas of potato cultivation in Romania, a constant trend of increasing air temperature and decreasing precipitation during the summer has been observed, especially in the flowering-maturing phenophase, when the plants achieve maximum water consumption (Stefan et al., 2024).

In this context, the aim of this paper is to present the effects of some biostimulators at potato crop according to variety and plant density.

MATERIALS AND METHODS

The performed field experiment was carried out in the years 2023 and 2024 at the National Institute of Research and Development for Potato and Sugar Beet Brașov (NIRDPSB Brașov), Romania, on a chermozem soil with a pH of 5.77.

The field experiment was organized as subdivided plots with 4 replications being of type 2 x 2 x 5, with the following experimental factors:

- Factor A – Potato variety, with two graduations:
  - a1. Darilena;
  - a2. Ultra.
- Factor B – Plant density, with two graduations:
  - b1. 45.000 plants/ha;
  - b2. 53.000 plants/ha.
- Factor C – Biostimulator product, with five graduations:
  - c1. No biostimulator – control variant;
  - c2. Genaktis 1;
  - c3. Irys;
  - c4. Terram Number One;
  - c5. Wuxal Macromix.

Darilena is a mid-early variety created by NIRDPSB Brașov, with oval tubers of yellow skin colour and light yellow flesh colour.

Ultra variety is a mid-early variety created by Solana Holland, with long oval tubers of yellow skin colour and light yellow flesh colour.

Genaktis 1 is a foliar biostimulator based on seaweed and silica extracts, with the effect of intensifying metabolic processes. It contains: 9% total nitrogen under the form of urea nitrogen, 5% P_2O_5 soluble in water, 7% K_2O soluble in water, 0.01% B soluble in water, 0.002% Cu soluble in water chelated with EDATA, 0.02% Fe, 0.001% Mo soluble in water. The biostimulant Genaktis 1 was applied twice during the vegetation period: the first application at the beginning of flowering and the second 15 days after the first application, at a rate of 3 l/ha/application.

Irys is a root biostimulator based on humic substances and plant extracts with a growth and development effect on the root system. It contains: 7% total nitrogen under the form of urea nitrogen, 9% P_2O_5 soluble in water, 13%

K_2O soluble in water, 0.01% B soluble in water, 0.002% Cu soluble in water chelated with EDATA, 0.04% Fe soluble in water chelated with EDATA, 0.01% Mn soluble in water chelated with EDATA, 0.001% Mo soluble in water, 0.002% Zn soluble in water chelated with EDATA. The biostimulant Irys was applied first at planting in the ridge and then at the three-differentiated-leaf stage, at a rate of 4 l/ha/application.

Terram Number One is a root biostimulator with high carbon content and organic substances, amino acids and polysaccharides. It contains: 1.2% total nitrogen under the form of soluble organic nitrogen, 12% organic carbon of biological origin, 7-8% K_2O, 35.5% organic substance with normal molecular weight <50 kDa, 7% alginic acid, 1.2-2.4% mannitol. The biostimulant Terram Number One was applied at a rate of 3 l/ha/application during the vegetation period, in two passes at 15-day intervals from the first application.

Wuxal Macromix is a foliar fertilizer with a biostimulating effect that has a well-balanced nutrient suspension indicated for sustaining plant growth under physiological stress conditions, having also a stabilizing effect on plant health. It contains: 16% N, 16% P_2O_5, 12% K_2O, 0.02% B, 0.05% Cu, 0.1% Fe, 0.05% Mn, 0.001% Mo, 0.05% Zn. The foliar fertilizer with a biostimulant effect on plants, Wuxal Macromix, was first applied at the beginning of tuber formation, with the second application 14 days after the first, at a rate of 5 l/ha/application.

In the spring of 2023 and 2024, NPK 15:15:15 fertilizers were applied at a rate of 1000 kg/ha, which were later incorporated into the soil during land preparation for planting. After ridge formation, the first herbicide application was performed using Sencor Liquid 600 SC (Metribuzin 600 g/l) at a rate of 0.6 l/ha for controlling annual dicotyledonous and monocotyledonous weeds, and Titus 25 DF (Rimsulfuron 25%) at a rate of 60 g/ha for controlling perennial grasses and certain susceptible broadleaf weeds. During the vegetation period, treatments were applied for pest control and preventive disease using a tractor equipped with a spraying pump.

The tubes were planted on 28th of April in 2023 and on 10th of April in 2024. The row spacing was of 75 cm.

The plot size for each variant was of 18 m^2, this resulting from four rows of potato plants at row spacing of 75 cm, which means 3 m, and 6 m along the rows.

The number of main stems and the weight of aerial part from two plants from each variant and replication were analysed.

On 21st of September in 2023 and 20th of September in 2024, the tubers were harvested and their weight from each plot and replication was determined, and then the yield per hectare was calculated.

The results were statistically analysed using ANOVA program (analysis of variance for fully randomized polyfactorial experiments).

Weather conditions during field research

In 2023, the month of April was rich in precipitation (83.1 mm), exceeding the multiannual amount by 33.1 mm, and the average temperature (7.3°C) was lower than the multiannual average (8.5°C). In May, the average temperature has almost the same value (13.8°C) as the multiannual average (13.6°C) and a relatively high amount of precipitation (77.5 mm) were recorded. The summer months (June-August) recorded average temperatures above the multiannual averages by 1.1°C, 2.4°C and 3.4°C respectively, and the precipitation level was higher in June with 14.4 mm and in August by 1.1 mm, but much lower in July (40.7 mm). In September, the average temperature was higher with 3.3°C and the amount of precipitation especially low, with only 11 mm (Table 1).

In 2024, the month of April was warmer than normal, and the amount of precipitation was 43.5 mm, lower by 6.5 mm compared to multiannual average. In May, the average temperature was at the level of the multiannual average, but the amount of precipitation was much lower than the multiannual average value, registering only 42.7 mm compared to 82 mm. The summer months (June-August) recorded higher average temperatures, with 4.6°C higher in June. Also, the volume of precipitation was much lower in all three months, setting in the phenomena of atmospheric and pedological drought. It should be emphasized that a large volume of precipitation fell in a very short time (for example, the month of August when 90% of the total quantity of the month fell in four

days). The month of September recorded an average temperature with 3.5°C higher than the multiannual average and a volume of precipitation of 57.8 mm (Table 1).

Table 1. Average air temperatures and rainfalls during the performed field experiments in the studied area

Year	Month						
	IV	V	VI	VII	VIII	IX	
Air temperature (°C)							Average
2023	7.3	13.8	17.6	20.5	20.9	16.9	16.2
2024	12.2	13.8	21.1	22.2	21.2	15.9	17.7
MAA*	8.5	13.6	16.5	18.1	17.5	13.6	14.6
Rainfalls (mm)							Amount
2023	83.1	77.5	111.1	59.1	77.5	11.0	419.3
2024	43.5	42.7	54.5	79.4	28.9	48.7	297.6
MAA*	50.0	82.0	96.7	99.8	76.4	52.5	457.4

*MAA - multiannual average

RESULTS AND DISCUSSIONS

Number of stems/hill

Regarding Darilena variety in 2023 (Table 2), at the first assessment (3rd of July, 2023), the number of stems/hill at the density of 45.000 plants/ha varied between 3.38 stems/hill to the control variant (untreated variant) and 5.38 stems/hill to the variant treated with Genaktis 1. There was recorded a significant difference only for the variant treated with Genaktis 1. At the same date but at density of 53.000 plants/ha the lowest number of stems/hill was to the control variant (3.00) and the highest again to the variant treated with Genaktis 1 (4.88), but there was not registered statistical differences.

At the second assessment (28th of July, 2023), the number of stems/hill at the density of 45.000 plants/ha varied between 4.13 stems/hill to the control variant and 5.13 stems to the variant treated with Terram Number One (Table 2). At the density of 45.000 plants/ha no treated variant determined significant differences compared to control one. At the same date but at the density of 53.000 plants/ha the lowest number of stems/hill was to the control variant (3.88) and the highest to the variant treated with Irys (5.38). At the densities of 53.000 plants/ha, the application of the biostimulators Irys and Wuxal Macromix determined statistically significant differences compared to control variant.

Table 2. Darilena cv. main stems/hill assessment in 2023

Experimental variant	Plant density of 45.000 pl/ha			Plant density of 53.000 pl/ha		
	Aver.	Dif.	Sign.	Aver.	Dif.	Sign.
No of main stems at 03rd of July, 2023						
Control	3.38	-	-	3.00	-	-
Genaktis 1	5.38	2.00	*	4.88	1.88	ns
Irys	4.25	0.88	Ns	3.63	0.63	ns
Terram Number One	4.38	1.00	Ns	3.63	0.63	ns
Wuxal Macromix	5.13	1.75	Ns	3.25	0.25	ns
	DL5% 1.92 DL1% 2.62 DL0.1% 3.56			DL5% 1.44 DL1% 1.97 DL0.1% 2.67		
No of main stems at 28th of July, 2023						
Control	4.13	-	-	3.88	-	-
Genaktis 1	4.25	0.13	ns	4.13	0.25	ns
Irys	4.63	0.50	ns	5.38	1.50	*
Terram Number One	5.13	1.00	ns	4.88	1.00	ns
Wuxal Macromix	4.50	0.38	ns	5.13	1.25	*
	DL5% 1.47 DL1% 2.01 DL0.1% 2.73			DL5% 1.14 DL1% 1.56 DL0.1% 2.11		

Regarding Darilena variety in 2024 (Table 3), at the first assessment (10th of June, 2024), the number of stems/hill at the density of 45.000 plants/ha varied between 4.25 to the control variant and 6.25 to the variant treated with Wuxal Macromix. At the same date but at the density of 53.000 plants/ha, the lowest number of stems/hill was to the control variant (4.00) and the highest was to the variant treated with Genaktis 1 (6.38). For both plant densities the variants treated with Genaktis 1 and Wuxal Macromix registered differences statistically significant compared to control (untreated) variant.

At the second assessment (17th of July, 2024), the number of stems/hill at the density of 45.000 plants/ha varied between 3.50 to the control variant and 5.63 to the variants treated with Genaktis 1 and Wuxal Macromix (Table 3). For this assessment at the density of 45.000 plants/ha, all the tested biostimulators determined differences statistically significant compared to control (untreated) variant.

At the same date but at the density of 53.000 plants/ha the lowest number of stems/hill was registered to the control variant (3.63) and the highest to the variants treated with Genaktis 1 and Terram Number One (6.13). At this plant density, the biostimulators Genaktis 1 and Terram Number

One determined differences statistically distinct significant compared to control variant, while the biostimulator Wuxal Macromix determined a difference statistically significand and the biostimulator Irys determined no significant differences.

Table 3. Darilena cv. main stems assessment in 2024

Experimental variant	Plant density of 45.000 pl/ha			Plant density of 53.000 pl/ha		
	Aver.	Dif.	Sign.	Aver.	Dif.	Sign.
No. of main stems at 10th of June, 2024						
Control	4.25	-	-	4.00	-	-
Genaktis 1	5.63	1.38	*	6.38	2.38	*
Irys	5.00	0.75	ns	4.75	0.75	ns
Terram Number One	5.38	1.13	ns	6.00	2.00	ns
Wuxal Macromix	6.25	2.00	*	6.13	2.13	*
	DL5% 1.33 DL1% 1.83 DL0.1% 2.48			DL5% 2.08 DL1% 2.85 DL0.1% 3.86		
No. of main stems at 17th of July, 2024						
Control	3.50	-	-	3.63	-	-
Genaktis 1	5.63	2.13	*	6.13	2.50	**
Irys	5.38	1.88	*	5.00	1.38	ns
Terram Number One	5.25	1.75	*	6.13	2.50	**
Wuxal Macromix	5.63	2.13	*	5.50	1.88	*
	DL5% 1.68 DL1% 2.30 DL0.1% 3.12			DL5% 1.44 DL1% 1.97 DL0.1% 2.68		

Regarding Ultra variety in 2023 (Table 4), at the first assessment (3rd of July, 2023), the number of stems/hill at the density of 45.000 plants/ha varied between 2.88 stems/hill to the untreated variant and 5.88 stems/hill to the variant treated with Genaktis 1. For the variant treated with Genaktis 1 there was recorded a very distinctly significant difference, while for variants treated with Irys and Wuxal Macromix distinctly significant difference and variant treated with Number One only a significant difference towards the control variant. At the same date but at the density of 53.000 plants/ha, the lowest number of stems/hill was to control (untreated) variant (3.75) and the highest to the variant treated with Irys (5.63), been recorded distinctly significant differences to the variants treated with Irys and Number One, and a significant difference to the variant treated with Wuxal Macromix.

At the second assessment (27th of July, 2023), the number of stems/hill at the density of 45.000 plants/ha varied between 4.5 to the control variant and 6.0 to the variant treated with Genaktis 1 (Table 1). Compared to control variant, a distinct significant difference was registered at the variant treated with Genaktis 1 and a significant one at the variant treated with Terram Number One. At the same date but at the density of 53.000 plants/ha, the lowest number of stems was registered to the control variant (4.25) and the highest to the variant treated with Irys (5.63). At this plant density, compared to control variant, the biostimualtors Irys and Wuxal Macromix determined differences statistically significant.

Table 4. Ultra cv. main stems assessment in 2023

Experimental variant	Plant density of 45.000 pl/ha			Plant density of 53.000 pl/ha		
	Aver.	Dif.	Sign.	Aver.	Dif.	Sign.
No. of main stems at 03rd of July, 2023						
Control	2.88	-	-	3.75	-	-
Genaktis 1	5.88	3.00	***	4.00	0.25	ns
Irys	4.75	1.88	**	5.63	1.88	**
Terram Number One	4.00	1.13	*	5.25	1.50	**
Wuxal Macromix	5.13	2.25	**	5.13	1.38	*
	DL5% 0.92 DL1% 1.26 DL0.1% 1.71			DL5% 1.07 DL1% 1.47 DL0.1% 1.99		
No. of main stems at 28th of July, 2023						
Control	4.50	-	-	4.25	-	-
Genaktis 1	6.00	1.50	**	5.25	1.00	ns
Irys	5.50	1.00	ns	5.63	1.38	*
Terram Number One	5.88	1.38	*	4.88	0.63	ns
Wuxal Macromix	4.88	0.38	ns	5.63	1.38	*
	DL5% 1.05 DL1% 1.43 DL0.1% 1.94			DL5% 1.27 DL1% 1.73 DL0.1% 2.35		

Regarding Ultra variety in 2024 (Table 5), at the first assessment (10th of June, 2024), the number of stems/hill to the density of 45.000 plants/ha varied between 4.13 stems/hill to the untreated variant and 6.13 stems/hill to the variant treated with Irys. Only the variants treated with Genaktis 1 and Irys recorded a distinctly significant difference towards the untreated variant. At the same date but at the density of 53.000 plants/ha, the lowest number of stems was to the control variant (4.0) and the highest to the variant treated with Number One (5.63), been recorded significant differences to variants treated with Genaktis 1, Number One and Wuxal Macromix.

At the second assessment (17th of July, 2024), the number of stems/hill at the density of 45.000 plants/ha varied between 4.13 to the control variant and 5.88 to the variant treated with Terram Number One, this variant being

the only one registering a difference statistically significant compared to control variant (Table 5). At the same date but at the density of 53.000 plants/ha, the lowest number of stems was to the control variant (4.25) and the highest to the variant treated with Genaktis 1 (6.00), this variant being the only one registering a difference statistically significant compared to control variant.

Table 5. Ultra cv. main stems assessment in 2024

Experimental variant	Plant density of 45.000 pl/ha			Plant density of 53.000 pl/ha		
	Aver.	Dif.	Sign.	Aver.	Dif.	Sign.
No. of main stems at 10th of June, 2024						
Control	4.13	-	-	4.00	-	-
Genaktis 1	5.88	1.75	**	5.50	1.50	*
Irys	6.13	2.00	**	4.75	0.75	ns
Terram Number One	4.38	0.25	ns	5.63	1.63	*
Wuxal Macromix	4.75	0.63	ns	5.50	1.50	*
	DL5% 1.23 DL1% 1.69 DL0.1% 2.29			DL5% 1.26 DL1% 1.72 DL0.1% 2.34		
No. of main stems at 17th of July, 2024						
Control	4.13	-	-	4.25	-	-
Genaktis 1	5.25	1.13	ns	6.00	1.75	*
Irys	5.38	1.25	ns	5.25	1.00	ns
Terram Number One	5.88	1.75	*	5.50	1.25	ns
Wuxal Macromix	5.63	1.50	ns	5.13	0.88	ns
	DL5% 1.60 DL1% 2.20 DL0.1% 2.98			DL5% 1.38 DL1% 1.89 DL0.1% 2.56		

Aerial part weight

Regarding Darilena variety in 2023 (Table 6), at the first assessment (3rd of July, 2023), the aerial part weight to the density of 45.000 plants/ha varied between 340.2 g to the control variant to 447.5 g to the variant treated with Terram Number One, to this variant been registered a very distinctly significant difference. At the same date but at the density of 53.000 plants/ha, the lowest weight was also to the control variant (219.6 g) and the highest to the variant treated with Genaktis 1 (391.7 g). At this plant density, the variant treated with Terram Number One registered a difference significant compared to control variant and very significant differences were registered to the variants treated with Genaktis 1 and Irys.

At the second assessment (28th of July, 2023), the aerial part weight at the density of 45.000 plants/ha varied between 305.7 g to the untreated variant and 433.4 g to the variant treated with Wuxal Macromix (Table 6). To this density there were recorded significant differences to variants treated with Genaktis 1 and Irys and a distinctly significant difference to the variant treated with Wuxal Macromix. At the same date but at the density of 53.000 plants/ha the lowest weight was to the control variant (325.7 g) and the highest to the variant treated with Irys (380.0 g) and were observed to all treated variants significant differences.

Table 6. Darilena cv. aerial part weight assessment in 2023

Experimental variant	Plant density of 45.000 pl/ha			Plant density of 53.000 pl/ha		
	Aver.	Dif.	Sign.	Aver.	Dif.	Sign.
Aerial part weight (g) at 03rd of July, 2023						
Control	340.2	-	-	219.6	-	-
Genaktis 1	342.0	1.75	ns	391.7	172.13	**
Irys	349.7	9.50	ns	367.7	148.13	**
Terram Number One	447.5	107.25	***	347.9	128.25	*
Wuxal Macromix	386.1	45.88	ns	317.0	97.38	ns
	DL5% 54.80 DL1% 74.99 DL0.1% 101.73			DL5% 101.6 DL1% 139.0 DL0.1% 188.60		
Aerial part weight (g) at 28th of July, 2023						
Control	305.7	-	-	325.7	-	-
Genaktis 1	386.1	80.38	*	373.0	47.25	*
Irys	389.0	83.25	*	380.0	54.25	*
Terram Number One	363.5	57.75	ns	371.5	45.75	*
Wuxal Macromix	433.4	127.63	**	376.5	50.75	*
	DL5% 75.63 DL1% 103.49 DL0.1% 140.40			DL5% 42.6 DL1% 58.3 DL0.1% 79.09		

Regarding Darilena variety in 2024 (Table 7), at the first assessment (10th of June, 2024), the aerial part weight at the density of 45.000 plants/ha varied between 217.75 g to the control variant and 309.75 g to the variant treated with Wuxal Macromix, to this variant being registered a very significant difference. Also a significant difference was recorded to the variant treated with Terram Number One and a distinctly significant one to the variant treated with Genaktis 1. At the same date but at the density of 53.000 plants/ha the lowest weight was also to the untreated variant (205.75 g) and the highest to the variant treated with Wuxal Macromix (268.0 g). At this plant density, a significant difference was registered at the variant treated with Irys, distinctly significant at the variant treated with Terram Number One and very significant differences at the variant treated with Wuxal Macromix.

At the second assessment (July, 17th, 2024), the aerial part weight to the density of 45.000 plants/ha varied between 139.75 g to the control variant and 201.88 g to the variant treated with Wuxal Macromix (Table 7). To this plant density, there were recorded very significant differences to variants treated with Wuxal Macromix and Genaktis 1 (195.5 g) and distinctly significant differences to variants treated with Irys (190.0 g) and Terram Number One (186.75 g). At the same date but at the density 53.000 plants/ha, the lowest weight was to the control variant (193.63 g) and the highest to the variant treated with Wuxal Macromix (241.25 g), where there were observed a distinctly significant difference as well as to the variant treated with Terram Number One, while the variants treated with Genaktis 1 and Irys registered no significant differences.

Table 7. Darilena cv. aerial part weight assessment in 2024

Experimental variant	Plant density of 45.000 pl/ha			Plant density of 53.000 pl/ha		
	Aver.	Dif.	Sign.	Aver.	Dif.	Sign.
Aerial part weight (g) at 10th of June, 2024						
Control	217.75	-	-	205.75	-	-
Genaktis 1	278.38	60.63	**	217.00	11.25	ns
Irys	235.50	17.75	ns	249.00	43.25	*
Terram Number One	254.50	36.75	*	259.13	53.38	**
Wuxal Macromix	309.75	92.00	***	268.00	62.25	***
	DL5% 32.96 DL1% 45.11 DL0.1% 61.20			DL5% 32.97 DL1% 45.12 DL0.1% 61.22		
Aerial part weight (g) at 17th of July, 2024						
Control	139.75	-	-	193.63	-	-
Genaktis 1	195.50	55.75	***	217.75	24.13	ns
Irys	190.00	50.25	**	220.00	26.38	ns
Terram Number One	186.75	47.00	**	234.50	40.88	**
Wuxal Macromix	201.88	62.13	***	241.25	47.63	**
	DL5% 28.52 DL1% 39.03 DL0.1% 52.95			DL5% 28.92 DL1% 39.57 DL0.1% 53.69		

Regarding Ultra variety in 2023 (Table 8), at the first assessment (3rd of July, 2023), the aerial part weight at the density of 45.000 plants/ha varied between 426.5 g to the untreated variant and 479.5 g to variant treated with Terram Number One, to this variant been registered a very significant difference compared to untreated variant, as well as at the variant treated with Genaktis 1. At the same date but at the density of 53.000 plants/ha, the lowest weight was also to the untreated variant (425.75 g) and the highest to the variant treated with Terram Number One (491.88 g). At this plant density, there were recorded a significant difference compared to control at the variant treated with Genaktis 1, and very significant differences to the variants treated with Terram Number One and Wuxal Macromix.

At the second assessment (28th July, 2024), the aerial part weight at the density of 45.000 plants/ha varied between 475.0 g to the untreated variant and 549.25 g to the variant treated with Number One, to this variant been recorded a very significant difference (Table 8). To variants treated with Genaktis 1 (529.25 g) and Wuxal Macromix (535.25 g), there were registered a distinctly significant difference, and to the variant treated with Irys (480.25 g) there was registered no significant difference. At the same date but at the density of 53.000 plants/ha, the lowest weight was to the control variant (409.13 g) and the highest to the variant treated with Wuxal Macromix (445.25 g), observing to all treated variants a significant difference compared to control variant.

Table 8. Ultra cv. aerial part weight assessment in 2023

Experimental variant	Plant density of 45.000 pl/ha			Plant density of 53.000 pl/ha		
	Aver.	Dif.	Sign.	Aver.	Dif.	Sign.
Aerial part weight (g) at 03rd of July, 2023						
Control	426.5	-	-	425.75	-	-
Genaktis 1	463.63	37.13	***	451.25	25.50	*
Irys	462.38	35.88	**	456.0	4.75	ns
Terram Number One	479.5	53.00	***	491.88	66.13	***
Wuxal Macromix	458.75	32.25	**	465.0	39.25	***
	DL5% 19.41 DL1% 26.56 DL0.1% 36.03			DL5% 19.62 DL1% 26.85 DL0.1% 36.43		
Aerial part weight (g) at 28th of July, 2023						
Control	475.00	-	-	409.13	-	-
Genaktis 1	529.25	54.25	**	442.00	32.88	*
Irys	480.25	5.25	ns	439.50	30.38	*
Terram Number One	549.25	74.25	***	439.50	30.38	*
Wuxal Macromix	535.25	60.25	**	445.25	36.13	*
	DL5% 38.26 DL1% 52.35 DL0.1% 71.02			DL5% 27.54 DL1% 37.68 DL0.1% 51.12		

Regarding Ultra variety in 2024 (Table 9), at the first assessment (10th of June, 2024), the aerial part weight at the density of 45.000 plants/ha varied between 251.75 g to the control variant and 355.75 g to the variant treated with Irys. To this variant and to the variants treated with Genaktis 1 (349.0 g) and

Wuxal Macromix (345.0 g), there were registered significant differences compared to control variant. At the same date but at the density of 53.000 plants/ha, the lowest weight was also registered to the untreated variant (206.50 g) and the highest to the variant treated with Irys (368.63 g). At this plant density, there were recorded a significant difference compared to control at the variant treated with Wuxal Macromix, distinctly significant differences to the variants treated with Genaktis 1 and Number One, and a very significant difference to the variant treated with Irys.

At the second assessment (17th of July, 2024), the aerial part weight at the density of 45.000 plants/ha varied between 145.88 g to the variant treated with Irys and 219.63 g to the variant treated with Number One (Table 9). To this plant density, there was recorded only a distinctly significant difference compared to control at the variant treated with Terram Number One. At the same date but at the density of 53.000 plants/ha, the lowest weight was registered to the control variant (199.25 g) and the highest to the variant treated with Genaktis 1 (306.25 g), observing a very significant differences to this one, and a significant difference to the variant treated with Wuxal Macromix compared to control variant.

Table 9. Ultra cv. aerial part weight assessment in 2024

Experimental variant	Plant density of 45.000 pl/ha			Plant density of 53.000 pl/ha		
	Aver.	Dif.	Sign.	Aver.	Dif.	Sign.
Aerial part weight (g) at 10th of June, 2024						
Control	251.75	-	-	206.50	-	-
Genaktis 1	349.00	97.25	*	310.38	103.88	**
Irys	355.75	104.00	*	368.63	162.13	***
Terram Number One	280.75	29.00	ns	302.00	95.50	**
Wuxal Macromix	345.00	93.25	*	286.25	79.75	*
	DL5% 88.05 DL1% 121.11 DL0.1% 164.30			DL5% 65.73 DL1% 89.94 DL0.1% 122.02		
Aerial part weight (g) at 17th of July, 2024						
Control	155.25	-	-	199.25	-	-
Genaktis 1	170.88	15.63	ns	306.25	107.00	***
Irys	145.88	-9.38	ns	251.25	52.00	ns
Terram Number One	219.63	64.38	**	239.00	39.75	ns
Wuxal Macromix	190.00	34.75	ns	279.25	80.00	*
	DL5% 38.81 DL1% 53.11 DL0.1% 72.05			DL5% 68.02 DL1% 93.08 DL0.1% 126.28		

Tuber yield

Regarding Ultra variety in 2023 (Table 10) and at the density of 45.000 plants/ha, the tuber yield ranged from 40.3 t/ha for the control variant to 46.4 t/ha, with the highest yield recorded for the variant treated with Wuxal Macromix. At the density of 53.000 plants/ha, the tuber yield values ranged between 42.5 t/ha for the control variant and 47.8 t/ha for the variant treated with Terram Number One. Comparing the two plant densities, it stands out that the Ultra variety showed highly significant positive differences compared to the untreated variant in all four variants treated with biostimulants at the density of 45.000 plants/ha, and at the density of 53.000 plants/ha, highly significant positive differences were recorded for the variants treated with Genaktis 1, Irys, and Number One (Table 10).

Regarding Ultra variety in 2024 (Table 10), the highest tuber yield at the density of 45.000 plants/ha was obtained in the variant treated with Irys (26.8 t/ha), compared to the control variant (24.3 t/ha). At a density of 53.000 plants/ha, the yield ranged between 25.6 t/ha (for control variant) and 28.4 t/ha for the variant treated with Terram Number One. Among the tested biostimulators, only Wuxal Macromix registered differences very significant compared to control at both plant densities. Genaktis 1 determined distinct significant differences at density of 45.000 plants/ha and very significant differences at density of 53.000 plants/ha. Iris determined very significant differences at density of 45.000 plants/ha and distinct significant differences at density of 53.000 plants/ha. Terram Number One determined no significant differences at density of 45.000 plants/ha and very significant differences at density of 53.000 plants/ha.

Analysing the two planting densities, it can be observed that the tuber yield was higher at the density of 53.000 plants/ha than those obtained at density of 45.000 plants/ha in both experimental years (Table 10).

Regarding Darilena variety in 2023 (Table 11) and at the density of 45.000 plants/ha, the tuber yield ranged between 26.1 t/ha for the untreated variant and 29.4 t/ha for the variant treated with Irys.

At the density of 53.000 plants/ha, the tuber yield ranged between 26.1 t/ha and 29.8 t/ha for the variant treated with Wuxal Macromix. Following the statistical analysis, very significant positive differences compared to the untreated variant at the two analysed densities were found in the variants treated with Genaktis 1 and Wuxal Macromix. The variant treated with Irys determined a difference very significant compared to control at density of 45.000 plants/ha and a difference distinct significant at density of 53.000 plants/ha. The variant treated with Terram Number One determined a difference significant compared to control at density of 45.000 plants/ha and a difference very significant at density of 53.000 plants/ha.

Table 10. Tuber yield at Ultra cv. (2023-2024)

Experimental variant	Plant density of 45.000 pl/ha			Plant density of 53.000 pl/ha		
	Aver.	Dif.	Sign.	Aver.	Dif.	Sign.
Tuber yield (t/ha) – 21.09.2023						
Control	40.3	-	-	42.5	-	-
Genaktis 1	43.8	3.45	***	46.1	3.58	***
Irys	44.4	4.05	***	45.0	2.49	***
Terram Number One	44.2	3.91	***	47.8	5.28	***
Wuxal Macromix	46.4	6.05	***	43.1	0.64	ns
Average	*43.8*	*3.5*	-	*44.9*	*2.4*	-
	DL5% 0.67 DL1% 0.90 DL0.1% 1.18			DL5% 0.84 DL1% 1.13 DL0.1% 1.48		
Tuber yield (t/ha) – 20.09.2024						
Control	24.3	-	-	25.6	-	-
Genaktis 1	25.9	1.57	**	27.4	1.87	***
Irys	26.8	2.43	***	27.2	1.63	***
Terram Number One	25.3	0.92	ns	28.4	2.81	***
Wuxal Macromix	26.6	2.23	***	28.0	2.45	***
Average	*25.8*	*1.5*	-	*27.3*	*1.7*	-
	DL5% 1.08 DL1% 1.44 DL0.1% 1.89			DL5% 0.83 DL1% 1.11 DL0.1% 1.45		

In 2024, the control variant presented a yield of 15.5 t/ha, while the variant treated with Wuxal Macromix (17.8 t/ha) had the highest yield at the density of 45.000 plants/ha (Table 11). At the density of 53.000 plants/ha, the yield was 16.1 t/ha for the control variant, with the highest yield of 18.6 t/ha for the variant treated with Wuxal Macromix. Following the statistical analysis, the variants treated with Irys and Wuxal Macromix recorded very significant positive differences compared to control at both plant densities.

According to potato variety, plant density and climatic conditions of the year, the yield increase determined by using of biostimulator products compared to control variant (with no biostimulator) varied between 0.64 and 6.5 t/ha (Tables 10 and 11). It has to be underlined that yield increase determined by using biostimulators was higher in the better climatic conditions of the year 2023 than in the drought year 2024 (Tables 10 and 11). Also, yield increase determined by using biostimulators was higher at the density of 53.000 plants/ha except the average tuber yield obtained at Ultra variety in 2023 (Tables 10 and 11).

Table 11. Total yield Darilena cv. (2023-2024)

Experimental variant	Plant density of 45.000 pl/ha			Plant density of 53.000 pl/ha		
	Aver.	Dif.	Sign.	Aver.	Dif.	Sign.
Tuber yield (t/ha) – 21.09.2023						
Control	26.1	-	-	26.1	-	-
Genaktis 1	28.6	2.49	***	28.3	2.20	***
Irys	29.4	3.28	***	27.6	1.50	**
Terram Number One	27.1	0.97	*	28.4	2.25	***
Wuxal Macromix	28.5	2.39	***	29.8	3.71	***
Average	*27.9*	*1.8*	-	*28.0*	*1.9*	-
	DL5% 0.90 DL1% 1.20 DL0.1% 1.58			DL5% 1.01 DL1% 1.35 DL0.1% 1.77		
Tuber yield (t/ha) – 20.09.2024						
Control	15.5	-	-	16.1	-	-
Genaktis 1	16.4	0.87	ns	17.6	1.56	*
Irys	17.5	1.94	***	18.4	2.31	**
Terram Number One	16.2	0.65	ns	16.8	0.79	ns
Wuxal Macromix	17.8	2.26	***	18.6	2.50	**
Average	*16.7*	*1.2*	-	*17.5*	*1.4*	-
	DL5% 1.02 DL1% 1.37 DL0.1% 1.80			DL5% 1.43 DL1% 1.91 DL0.1% 2.51		

The tuber yields obtained in the experimental field in 2024 were much lower compared to 2023 (Figure 1). An important cause of lower production in 2024 is the deficit of water in the soil during the growth of plants and tubers and the uneven distribution of precipitation during the vegetation period.

As average values for the whole experiment obtained in the experimental variants treated with biostimulators, all the used biostimulator products determined increases of tuber yield compared to control variant varying from 2.2 to 2.79 t/ha (Figure 1). Also as average values, the tuber yields were higher at density of 53.000 plants/ha, and Ultra variety proved to be superior to Darilena variety (Figure 1).

CONCLUSIONS

The better climatic conditions of the year 2023, with moderate thermo-hydric stress make the potato plants to be more vigorous which leaded to higher tuber yields, regardless of variety and plant density, compared to the year 2024 when the potato crop experienced severe drought.

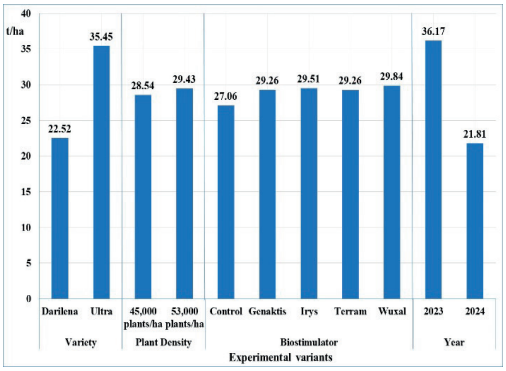


Figure 1. Average values of the tuber yield for the experimental variants

It is obvious that stem number is influenced by cultivars and potato tubers size, but all the used biostimulator products determined an increased number of stems per hill compared to control (untreated) variant. Also, all the used biostimulator products determined an increased aerial part weight compared to control variant.
By using biostimulator products, the tuber yield increased compared to control regardless the potato variety, plant density and climatic conditions of the year, the yield increase varying between 0.64 and 6.5 t/ha. The yield increase determined by using biostimulators is higher in better climatic conditions and at higher plant density. Having all these into account, the inclusion of the biostimulators in the potato crop technology can be considered a good strategy.

ACKNOWLEDGEMENTS

Data presented in this paper are part of research PhD programme of the first author within the University of Agronomic Sciences and Veterinary Medicine of Bucharest. Authors are thankful to NIRDPSB Brasov for supporting the performed research.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

SUNFLOWER PRODUCTION OF SOME GENOTYPES IN YEARS 2022, 2023 AND 2024, IN ROMANIA

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Abstract

In this paper we present results about seed yield of some sunflower genotypes in the period 2022-2024, in nine counties from Romania. Three genotypes belonging to NARDI Fundulea were analysed, two experimental sunflower hybrids HS 1112 and HS9233 and one commercial sunflower hybrid FD15E27. In year 2023 was registered the best average seed yield of all three sunflower hybrids of 3189 kg/ha followed by year 2022 with 2930 kg/ha and the lowest average seed yield was registered in year 2024 with 2310kg/ha. Experimental sunflower hybrid HS9233 which is suitable for Clearfield Plus system, registered the highest seed yield of 3045 kg/ha in year 2022 and 3241 kg/ha in year 2023. In year 2024, commercial sunflower hybrid FD15E27 registered the highest seed yield with 2417 kg/ha which is suitable for Express system. The agricultural year 2024 remains representative for the sunflower culture regarding lowest seed yield, because the climate changes were most evident through very high temperatures during the summer.

Key words*: sunflower, seed yield, drought, extreme heat.*

INTRODUCTION

In Romania in last years, sunflower (*Helianthus annuus* L.) is main oil crop and were cultivated on surface over 1 million hectares (Soare & Chiurciu, 2023). In 2022 were cultivated on 1.093 million hectares with an average seed yield of 1927 kg/ha and total production of 2.1 million tons. In 2023 were cultivated on 1.077 million hectares with an average seed yield of 1870 kg/ha and total production of 2.0 million tons. In 2024 were cultivated on 1.332 million hectares with an average seed yield of 993 kg/ha and total production of 1.3 million tons (MADR, Revista Ferma, Economica, EUROSTAT). Although sunflower has tolerance to water and heat stress conditions (Agüera & de la Haba, 2021; Markulj Kulundžić et al., 2022), seed yield of sunflower is affected by heat and drought (Anton et al., 2024; Beteri et al., 2024; Gurkan et al., 2021). In sunflower culture, high temperatures affect pollen viability and pollination (Astiz & Hernández, 2013; Mehmood et al., 2023; Moroldo et al., 2024). In sunflower culture, climate changes thought variation in temperature and precipitation affect directly production and period of vegetation (Gurkan, 2023; Popescu et al., 2023). Sunflower production is depending on climatic conditions such temperature and precipitations (Cojocaru et al., 2023; Partal, 2022).

MATERIALS AND METHODS

In period 2022-2024, we tested three sunflower hybrids, HS 1112, HS 9233 and FD 15E27 belonging to NARDI Fundulea, in nine counties, Dolj, Bihor, Braila, Vaslui, Timis, Buzau, Telorman, Galati and Calarasi from Romania.
HS1112 is experimental oil sunflower hybrid for and is suitable for conventional system and for organic system. HS 9233 is experimental oil sunflower hybrid resistant at Imidazoline herbicides and is suitable for Clearfield Plus system. FD15E27 is commercial oil sunflower hybrid resistant at sulfonylurea herbicides and is suitable for Express system.

RESULTS AND DISCUSSIONS

Average annual temperature on country was in Romania, 10.64^0C in year 2022, 11.38^0C in year

2023 and 11.62^{0}C in year 2024 (Figure 1). In terms of the average annual temperature ^{0}C in Romania, 2024 was the warmest in the last 60 years, followed by 2023 and 2022 was in fifth place (Table 1).

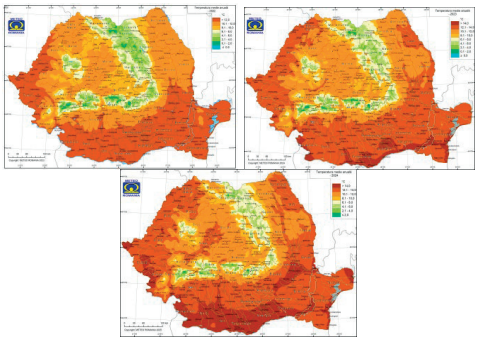


Figure 1. Average annual temperature in years 2022, 2023 and 2024, in Romania (source: www.meteoromania.ro)

In terms of the average annual temperature ^{0}C in Romania, 2024 was the warmest in the last 60 years, followed by 2023 and 2022 was in fifth place (Table 1). Rising temperatures can affect the growth and yield of sunflowers. Sunflowers thrive in warm conditions, but excessively high temperatures can lead to heat stress, reducing seed yield.

Table 1. Average annual temperature in Romania (source: www.meteoromania.ro)

Top hottest years	Year	Average annual temperature (°C)
1	**2024**	11.62
2	**2023**	11.38
3	2019	11.00
4	2020	10.78
5	**2022**	10.64
6	2015	10.58
7	2018	10.53
8	2007	10.48
9	2014	10.35
10	1994	10.24

Total average of rainfalls on country registered in Romania in year 2022 was 553.2 mm, in year 2023 was 661.3 mm and in year 2024 was 551.4 mm (Figure 2). Regarding rainfalls, years 2022 and 2024 are characterized as drought years and 2023 as normal. Changes in rainfall patterns can result in droughts. Sunflowers require a certain amount of water for optimal growth, so drought can affect production and can lead to reduced seed size and can lead to reduced sunflower seed yield.

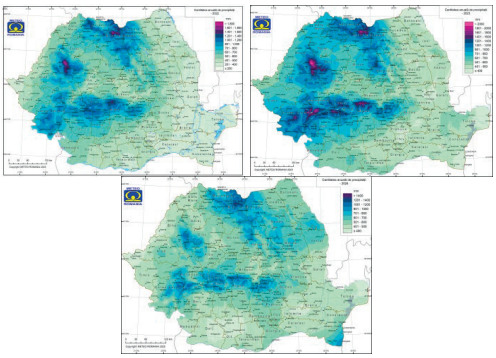


Figure 2. Total average rainfalls registered in years 2022, 2023 and 2024 in Romania (source: www.meteoromania.ro)

Availability and management of water resources are crucial, especially in regions prone to drought.

Variability in rainfall, temperature, and sunlight can significantly impact sunflower crop growth.

In year 2022, average seed yield on counties was between 1674 kg/ha in Bihor and 3721 kg/ha in Galati (Table 2). In year 2022, average seed yield of sunflower hybrids was between 2735 kg/ha at HS1112 and 3045 kg/ha at HS9233.

Table 2. Average seed yield of sunflower genotypes registered in year 2022, in nine counties from Romania

County	Average seed yield (kg)			
	HS1112	HS9233	FD15E27	Average
Dolj	3364	3979	3545	3629
Bihor	1605	1760	1659	1674
Braila	1281	2571	1779	1877
Vaslui	2498	3092	3785	3125
Timis	2373	2135	2322	2276
Buzau	3486	3267	2941	3231
Telorman	3288	3293	3690	3423
Galati	3326	3848	3991	3721
Calarasi	3395	3465	3380	3413
Average	2735	3045	3010	2930

In year 2022, one thousand seed weight (TSW g) on counties was between 45 g in Bihor and 66 g in Galati (Table 3). In year 2022, average one thousand seed weight (TSW g) of sunflower hybrids was between 52 g at FD15E27 and 64 g at HS9233.

Table 3. Average one thousand seed weight (TSW g) of sunflower genotypes registered in year 2022, in nine counties from Romania

County	TSW (g)			
	HS1112	HS9233	FD15E27	Average
Dolj	63	80	50	64
Bihor	39	50	46	45
Braila	71	72	54	65
Vaslui	54	61	47	54
Timis	52	61	61	58
Buzau	52	66	57	58
Telorman	52	62	50	54
Galati	71	72	56	66
Calarasi	60	53	55	56
Average	57	64	52	57

In year 2022, hectolitre weight (HW kg/hl) on counties was between 36 kg/hl in Calarasi and 42 kg/hl in Telorman (Table 4). In year 2022, average hectolitre weight (HW kg/hl) of sunflower hybrids was between 38 kg/hl at FD15E27, HS9233 and 40 kg/hl at HS1112.

Table 4. Average hectolitre weight (HW kg/hl) of sunflower genotypes registered in year 2022, in nine counties from Romania

County	Hectolitre weight (kg/hl)			
	HS1112	HS9233	FD15E27	Average
Dolj	40	39	40	39
Bihor	39	38	40	39
Braila	42	39	36	39
Vaslui	39	39	39	39
Timis	39	39	39	39
Buzau	40	38	38	38
Telorman	43	42	43	42
Galati	44	40	38	40
Calarasi	36	35	37	36
Average	40	38	38	39

In year 2022, average height (cm) of sunflower hybrids was between 133 cm at HS1112 and 143 cm at HS9233. In year 2022, height (cm) on counties was between 106 cm in Bihor and 168 cm in Dolj (Table 5).

Table 5. Average height (cm) of sunflower genotypes registered in year 2022, in nine counties from Romania

County	Height (cm)			
	HS1112	HS9233	FD15E27	Average
Dolj	165	175	165	168
Bihor	113	110	95	106
Braila	114	162	135	137
Vaslui	150	160	137	149
Timis	104	116	147	122
Buzau	125	130	125	126
Telorman	147	140	133	140
Galati	138	160	147	148
Calarasi	145	135	160	146
Average	133	143	138	138

In year 2022, average period of vegetation (days) of sunflower hybrids was between 115 days at HS1112 and FD15E27 and 116 days at HS9233. In year 2022, period of vegetation (days) on counties was between 108 days in Timis and 127 days in Braila (Table 6).

Table 6. Average period of vegetation (days) of sunflower genotypes registered in year 2022, in nine counties from Romania

County	Period of vegetation (days)			
	HS1112	HS9233	FD15E27	Average
Dolj	113	113	113	113
Bihor	117	117	117	117
Braila	126	130	126	127
Vaslui	120	120	118	119
Timis	108	108	108	108
Buzau	122	121	117	120
Telorman	108	111	109	109
Galati	121	122	122	121
Calarasi	106	109	113	109
Average	115	116	115	115

In year 2023, average seed yield on counties were between 2242 kg/ha in Vaslui and 4056 kg/ha in Galati (Table 7). In year 2023, average seed yield of sunflower hybrids was between 3104 kg/ha at FD15E27 and 3241 kg/ha at HS9233.

Table 7. Average seed yield of sunflower genotypes registered in year 2023, in nine counties from Romania

County	Average seed yield (kg)			
	HS1112	HS9233	FD15E27	Average
Dolj	3483	3318	3316	3372
Bihor	3472	3737	3457	3555
Braila	3053	3422	2520	2998
Vaslui	2056	2169	2501	2242
Timis	3602	3273	3466	3447
Buzau	3190	3307	2887	3128
Telorman	2409	2242	2492	2381
Galati	4588	4057	3523	4056
Calarasi	3152	3647	3774	3524
Average	3222	3241	3104	3189

In year 2023, one thousand seed weight (TSW g) on counties was between 39 g in Teleorman and 65 g in Timis (Table 8). In year 2023, average one thousand seed weight (TSW g) of sunflower hybrids was between 50 g at HS1112 and 65 g at HS9233.

Table 8. Average one thousand seed weight (TSW g) of sunflower genotypes registered in year 2023, in nine counties from Romania

County	TSW (g)			
	HS1112	HS9233	FD15E27	Average
Dolj	64	60	45	56
Bihor	45	64	52	53
Braila	54	72	57	61
Vaslui	58	54	74	62
Timis	47	84	66	65
Buzau	48	64	55	55
Telorman	32	46	41	39
Galati	54	70	57	60
Calarasi	51	72	65	62
Average	50	65	56	57

In year 2023, hectolitre weight (HW kg/hl) on counties was between 36 kg/hl in Vaslui and Buzau and 42 kg/hl in Timis and Calarasi (Table 9). In year 2023, average hectolitre weight (HW kg/hl) of sunflower hybrids was between 38 kg/hl at HS9233 and 39 kg/hl at HS1112 and FD15E27.

Table 9. Average hectolitre weight (HW kg/hl) of sunflower genotypes registered in year 2023, in nine counties from Romania

County	Hectolitre weight (kg/hl)			
	HS1112	HS9233	FD15E27	Average
Dolj	40	39	41	40
Bihor	41	39	39	39
Braila	39	39	38	38
Vaslui	36	36	37	36
Timis	44	39	43	42
Buzau	38	36	36	36
Telorman	39	40	43	40
Galati	38	39	37	38
Calarasi	42	42	42	42
Average	39	38	39	39

In year 2023, average height (cm) of sunflower hybrids was between 131 cm at HS1112 and 168 cm at HS9233. In year 2023, height (cm) on counties was between 121 cm in Braila and 181 cm in Dolj (Table 10).

Table 10. Average height (cm) of sunflower genotypes registered in year 2023, in nine counties from Romania

County	Height (cm)			
	HS1112	HS9233	FD15E27	Average
Dolj	155	215	175	181
Bihor	134	157	153	148
Braila	100	140	125	121
Vaslui	134	170	161	155
Timis	155	193	173	173
Buzau	120	160	135	138
Telorman	127	156	145	142
Galati	140	180	165	161
Calarasi	119	158	130	135
Average	131	168	151	150

In year 2023, average period of vegetation (days) of sunflower hybrids was between 105 days at HS1112 and 107 days at HS9233. In year 2023, period of vegetation (days) on counties was between 95 days in Teleorman and 131 days in Braila (Table 11).

Table 11. Average period of vegetation (days) of sunflower genotypes registered in year 2023, in nine counties from Romania

County	Period of vegetation (days)			
	HS1112	HS9233	FD15E27	Averag e
Dolj	102	105	102	103
Bihor	111	111	111	111
Braila	133	131	131	131
Vaslui	97	100	98	98
Timis	99	103	104	102
Buzau	116	115	115	115
Telorman	92	97	97	95
Galati	100	100	100	100
Calarasi	102	104	103	103
Average	105	107	106	106

In year 2024, average seed yield on counties were between 1137 kg/ha in Teleorman and 3134 kg/ha in Timis (Table 12). In year 2024, average seed yield of sunflower hybrids was between 2162 kg/ha at HS2162 and 24171 kg/ha at FD15E27.

Table 12. Average seed yield of sunflower genotypes registered in year 2024, in nine counties from Romania

County	Average seed yield (kg)			
	HS1112	HS9233	FD15E27	Average
Dolj	1412	1891	2158	1820
Bihor	2000	2178	2115	2097
Braila	2932	3258	2990	3060
Vaslui	2671	2959	2654	2761
Timis	3095	3088	3220	3134
Buzau	1620	1819	1629	1689
Telorman	1089	1082	1242	1137
Galati	2353	2677	2883	2637
Calarasi	2292	2224	2865	2460
Average	2162	2352	2417	2310

In year 2024, one thousand seed weight (TSW g) on counties was between 30 g in Teleorman and 63 g in Timis (Table 13). In year 2024, average one thousand seed weight (TSW g) of sunflower hybrids was between 42 g at HS1112 and 53 g at HS9233.

Table 13. Average one thousand seed weight (TSW g) of sunflower genotypes registered in year 2024, in nine counties from Romania

County	TSW (g)			
	HS1112	HS9233	FD15E27	Averag e
Dolj	43	57	54	51
Bihor	38	46	46	43
Braila	48	70	56	58
Vaslui	41	43	46	43
Timis	63	58	68	63
Buzau	37	42	37	38
Telorman	25	32	33	30
Galati	44	55	56	51
Calarasi	46	75	63	61
Average	42	53	51	48

In year 2024, hectolitre weight (HW kg/hl) on counties was between 35 kg/hl in Bihor and Telorman and 41 kg/hl in Dolj and Timis (Table 14). In year 2024, average hectolitre weight (HW kg/hl) of sunflower hybrids was between 37 kg/hl at HS1112 and HS9233 and 39 kg/hl at FD15E27.

Table 14. Average hectolitre weight (HW kg/hl) of sunflower genotypes registered in year 2024, in nine counties from Romania

County	Hectolitre weight (kg/hl)			
	HS1112	HS9233	FD15E27	Average
Dolj	41	42	41	41
Bihor	37	34	36	35
Braila	39	40	41	40
Vaslui	37	37	39	37
Timis	38	42	43	41
Buzau	38	35	36	36
Telorman	33	35	39	35
Galati	37	38	40	38
Calarasi	38	38	43	39
Average	37	37	39	38

In year 2024, average height (cm) of sunflower hybrids was between 129 cm at HS1112 and 149 cm at HS9233. In year 2024, height (cm) on counties was between 95 cm in Teleorman and 175 cm in Vaslui (Table 15).

Table 15. Average height (cm) of sunflower genotypes registered in year 2024, in nine counties from Romania

County	Height (cm)			
	HS1112	HS9233	FD15E27	Average
Dolj	120	175	170	155
Bihor	90	112	104	102
Braila	144	174	147	155
Vaslui	160	185	180	175
Timis	163	139	141	147
Buzau	162	150	140	150
Telorman	93	90	102	95
Galati	115	160	120	131
Calarasi	115	162	135	137
Average	129	149	137	138

In year 2024, average period of vegetation (days) of sunflower hybrids was between 113 days at HS1112 and HS9233 and 114 days at FD15E27. In year 2024, period of vegetation (days) on counties was between 96 days in Galati and 128 days in Braila (Table 16).

Table 16. Average period of vegetation (days) of sunflower genotypes registered in year 2024, in nine counties from Romania

County	Period of vegetation (days)			
	HS1112	HS9233	FD15E27	Average
Dolj	104	106	111	107
Bihor	123	123	124	123
Braila	128	128	128	128
Vaslui	112	113	117	114
Timis	108	110	114	110
Buzau	118	118	114	116
Telorman	105	105	104	104
Galati	97	96	97	96
Calarasi	123	123	122	122
Average	113	113	114	113

In period 2022-2024, average seed yield of sunflower hybrids in nine counties from Romania were between 2706 kg/ha at HS1112 and 2879 kg/ha at HS9233 (Table 17). In year 2023, average seed yield of sunflower hybrids was the highest with 3189 kg/ha and year 2024 was the lowest with 2310 kg/ha.

Table 17. Average seed yield of sunflower genotypes registered in years 2022, 2023 and 2024

Sunflower genotype	Average seed yield (kg)			
	2022	2023	2024	Average
HS1112	2735	3222	2162	2706
HS9233	3045	3241	2352	2879
FD15E27	3010	3104	2417	2843
Average	**2930**	**3189**	**2310**	2809

Counties significantly influences seed yield, while genotype does not (Figure 3). Differences between sunflower genotypes are not statistically significant. The year significantly influences seed yield, meaning the differences in yield between 2022, 2023 and 2024 are statistically significant.
Environmental explain variations between counties and climate strongly influence seed yield.

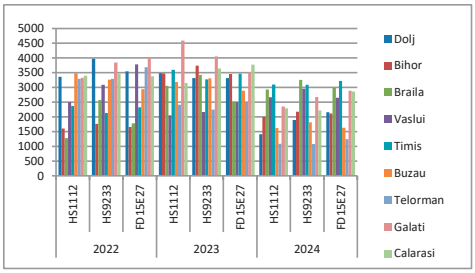


Figure 3. Average seed yield of sunflower genotypes registered in years 2022, 2023 and 2024 in nine counties from Romania

After analyzing the production of sunflower hybrids in nine counties, we created a map with the favorable areas for sunflower culture (Figure 4).

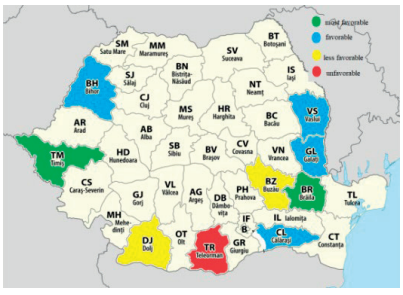


Figure 4. Favorable areas for sunflower culture in Romania

The most stable average hectolitre weight was at sunflower hybrid HS9233 in all years of studies (Figure 5). In year 2024 that was the hottest from last 60 years, the highest average hectolitre weight was registered by sunflower hybrid FD15E27.

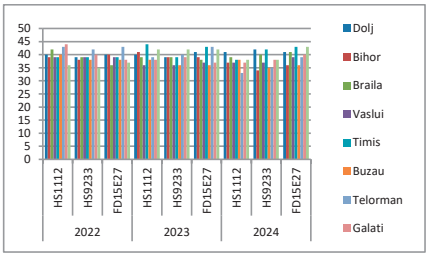


Figure 5. Average hectolitre weight (HW kg/hl) of sunflower genotypes registered in period 2022-2024

The highest average one thousand seed weight (TSW g) was registered by sunflower hybrid HS9233 in period 2022-2024 (Figure 6). Is a positive correlation between TSW and seed yield. If TSW increase, seed yield increase because heavier seeds lead to greater seed production.

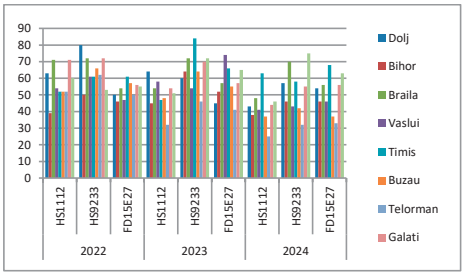


Figure 6. Average one thousand seed weight (TSW g) of sunflower genotypes registered in period 2022-2024

Average plant height (cm) was variable each year and in each county (Figure 7) with the highest records in 2023.

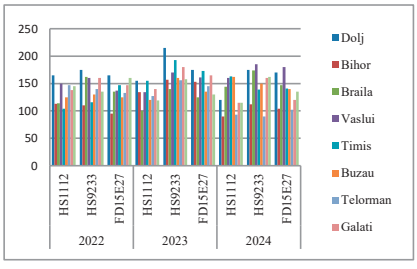


Figure 7. Average plant height (cm) of sunflower genotypes registered in period 2022-2024, in nine counties from Romania

In county Braila was registered the highest period of vegetation in all three years of study (Figure 8).



Figure 8. Average period of vegetation (days) of sunflower genotypes registered in period 2022-2024

Factors county (A), year (B), sunflower hybrid (C) and interaction between these factors influenced very significant positive seed yield (kg/ha), one thousand seed weight (TSW g), hectolitre weight (HW kg/hl) and plant height (cm) of sunflower hybrids (Tables 18-21).

Table 18. ANOVA analysis of variance for seed yield (kg/h) of sunflower hybrids in nine locations from Romania, in years 2022, 2023 and 2024

Source	Degrees of Freedom	Sum of Squares	Mean Square	F value	Signification
Factor A	8	27790874.519	3473859.315	310.8284	***
Factor B	2	31245466.691	15622733.346	1397.8659	***
AB	16	72924148.346	4557759.272	407.8119	***
Error	52	581158.864	11176.132		
Factor C	2	1356078.321	678039.160	33.6622	***
AC	16	4853226.494	303326.656	15.0591	***
BC	4	1284069.432	321017.358	15.9374	***
ABC	32	6024126.864	188253.965	9.3461	***
Error	108	2175384.222	20142.447		

Table 19. ANOVA analysis of variance for one thousand seed weight (TSW g) of sunflower hybrids in nine locations from Romania, in years 2022, 2023 and 2024

Source	Degrees of Freedom	Sum of Squares	Mean Square	F value	Signification
Factor A	8	7314.346	914.293	1362.7045	***
Factor B	2	2758.753	1379.377	2055.8861	***
AB	16	3525.802	220.363	328.4386	***
Error	26	17.444	0.671		
Factor C	2	3181.123	1590.562	606.2847	***
AC	16	13183765	82.423	31.4176	***
BC	4	985.802	246.451	93.9412	***
ABC	32	3193.975	99.812	38.0459	***
Error	54	141.667	2.623		

Table 20. ANOVA analysis of variance for hectolitre weight (HW kg/hl) of sunflower hybrids in nine locations from Romania, in years 2022, 2023 and 2024

Source	Degrees of Freedom	Sum of Squares	Mean Square	F value	Signification
Factor A	8	180.494	22.562	101.5278	***
Factor B	2	33.975	16.988	76.4444	***
AB	16	383.802	23.988	107.9444	***
Error	26	5.778	0.222		
Factor C	2	29.827	14.914	67.1111	***
AC	16	98.617	6.164	27.7361	***
BC	4	57.580	14.395	64.7778	***
ABC	32	127.309	3.978	17.9028	***
Error	54	12.000	0.222		

Table 21. ANOVA analysis of variance for plant height (cm) of sunflower hybrids in nine locations from Romania, in years 2022, 2023 and 2024

Source	Degrees of Freedom	Sum of Squares	Mean Square	F value	Signification
Factor A	8	35195.383	4399.423	1140.0498	***
Factor B	2	5717.049	2858.525	740.7473	***
AB	16	32755.951	2047.247	530.5158	***
Error	26	100.333	3.859		
Factor C	2	14538.235	7269.117	1872.1733	***
AC	16	4896.099	306.006	78.8124	***
BC	4	3824.321	956.080	246.2401	***
ABC	32	10328.346	322.761	83.1276	***
Error	54	209.667	3.883		

Factor A - County; Factor B - Year; Factor C - Sunflower hybrid; AB - Interaction between Factor County and Factor Year; AC - Interaction between Factor County and Factor Sunflower hybrid; BC - Interaction between Factor Year and Factor Sunflower hybrid; ABC - Interaction between Factor County and Factor Year and Factor Sunflower hybrid

CONCLUSIONS

The agricultural year 2023 was the most suitable for sunflower culture and year 2024 was the most unfavourable for sunflower culture do to extreme heat registered for a long period of time especially in period of flowering and seed filing. There are fluctuations in yields across the years, indicating varying agricultural conditions, including climate factors. Long-term shifts in weather patterns can alter growing seasons and affect the viability of sunflower crop in specific regions.

Different sunflower hybrid may have varying resilience to environmental stressors. The choice of cultivar can influence yield stability.

Experimental sunflower hybrid HS9233, suitable for Clearfield Plus system were the most adapted for climate changes followed by sunflower hybrid FD15E27.

This data highlights the importance of monitoring agricultural yields over time to understand trends, challenges and opportunities for improvement in sunflower seed production.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

TILLAGE AND NITROGEN RATE EFFECTS ON MAIZE IN THE SPECIFIC GROWING CONDITIONS OF DANUBE MEADOW

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Abstract

Tillage is an important tool within the crop technology to ensure good growing conditions for maize plants, decrease production costs, and improve soil characteristics. Also, nitrogen has a significant effect on crops, especially when it comes to maize, this being the most important nutrient that affects plant growth and yielding capacity of the plants. Thus, the paper aims to present the effects of different tillage and nitrogen rates on maize grain yield and yielding elements in the specific growing conditions of Danube Meadow from South Romania. In this respect, the research was performed in the years 2023 and 2024, in field experiments under rainfed conditions located in the Danube Meadow from South Romania, respectively near Oltenița city from Călărași County. The experimental factors were tillage methods (Plowing at 25 cm + 2 disc harrows passes; Scarifying at 35 cm + 2 disc harrows passes; Gruber Tiger at 25 cm; Gruber Tiger at 15 cm; Disc harrow at 15 cm x 2 passes) and nitrogen rate (0, 80, 120, and 160 kg/ha). Nitrogen fertilization had a positive effect on maize yielding elements and grain yield, especially at the highest rate regardless of the tillage method or climatic conditions of the year. The highest average grain yields were obtained in the case of variant of tillage with Gruber Tiger at 25 cm depth, especially in the better climatic conditions and associated with high nitrogen rate (160 kg/ha).

Key words*: maize, tillage, nitrogen rate, grain yield, yielding elements.*

INTRODUCTION

Due to constantly changing climatic conditions, the individual requirements of crop species and technical progress, soil cultivation methods have constantly evolved. Moreover, the sustainable development of agriculture has stated that there is no universally applicable system for tillage because of the local differences, especially climate and soil type and also the technical level of endowment (Rusu et al., 2009). A single tillage system is not suitable for all soils and climatic conditions, and therefore, the choice of the best suited tillage system must be harmonious to the particular agro-ecological environment (Barut & Akbolat, 2005). Appropriate tillage systems are soil - and crop-specific and their adaptation is governed by both biophysical and socio-economic factors (Lai, 1991). Practically, a specific tillage system is depending on the crop and cultivation conditions.

Within the crop technologies, tillage represents an important element and therefore it is necessary to be carried out in the best conditions (Khan et al., 2021). Therefore, farmers must know some particularities and type of soil, the presence of problematic weeds, and some characteristics of the crops in order to develop tillage methods, the necessary equipment and execution indices (Carr et al., 2013). Moreover, reality of climate changes requires a careful revision of traditional soil and plant management technologies (Țopa et al., 2010).

Tillage is one of the most energy-intensive processes in the agricultural production (Stajnko et al., 2009). In addition to the classic tillage system that includes ploughing, in order to save energy embedded in field crop technology and to avoid repeated passes with tractors and agricultural machinery, with negative effects on the soil, more and more farmers are interested in alternative tillage system.

There are many types of tillage systems such as conventional tillage, conservation tillage, minimum tillage, reduced tillage and no tillage systems (Siemens et al., 1992; Shah et al., 2016).

The tillage system is an essential maize-growing practice for successful production (Chețan et al., 2023).
The application of the most advanced crop technologies is taken into account using larger quantities of fertilizers, insecticides, fungicides and herbicides, and impose knowledge of the particularities of the crops and the pedoclimatic conditions of the area where work is being done (Cionca et al., 2024).
On a global scale, nitrogen is the most used fertilizer nutrient in agriculture. Studies have shown that cultivated plant species use only about 50% of applied N effectively, while the rest is lost through various pathways to the environment (Govindasamy et al., 2023). The selection of the right nitrogen fertilizer product, the appropriate rate, time and method of application are essential for farmers (Velicu & Ion, 2024). The current climatic changes require from farmers to optimize nitrogen fertilization according to the specific growing conditions of their crops.
Fertilization on maize crops is a very important technological issue that significantly contributes to increased productivity. Nitrogen is considered a limiting macronutrient for maize plants, this being the nutrient the plants need the most. The growth and the development of maize plant are very much affected by nitrogen; therefore, nitrogen fertilizer's optimal use is essential (Fathi & Zeidali, 2021).
The aim of the performed research was to investigate the effects of different tillage and nitrogen rates on maize grain yield and yielding elements in the specific growing conditions of Danube Meadow from South Romania.

MATERIALS AND METHODS

Research was performed in the years 2023 and 2024, in field experiments under rainfed conditions located in the Danube Meadow from South Romania, respectively near Oltenița city from Călărași County.
The relief in the area of research bears the imprint of meadow characteristics, this being characterized by a horizontal plane, with a land slope between 0.5 and 2%.
The soil is of alluvial type, and has a medium to heavy texture, an upper horizon of 20-35 cm thick, a dark brown - yellowish colour, and a granular structure.
The temperature values recorded in both 2023 and 2024 are notable for exceeding values in summer time by over 2-3ºC compared to the average for the area (27ºC) (Figure 1). The maximum recorded value was 42.2ºC on August 2024, and the minimum recorded value was -2.9ºC on February 17, 2023. The year 2024 was warmer than the year 2023, especially in the summer and winter seasons.
Regarding rainfall, there was a major water deficit in February, but a rainy period in April, while the summer months had small rainfall (Figure 2). From May to July, when maize plants are in the most sensitive period to water stress, the year 2024 was droughter than the year 2023.

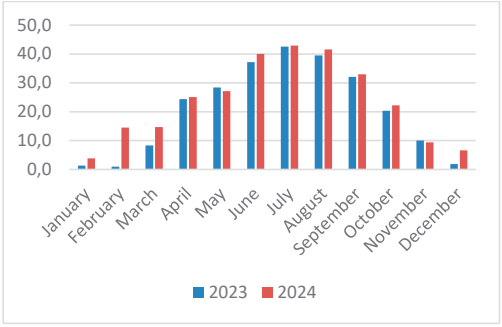


Figure 1. Temperatures in experimental field (2023-2024)

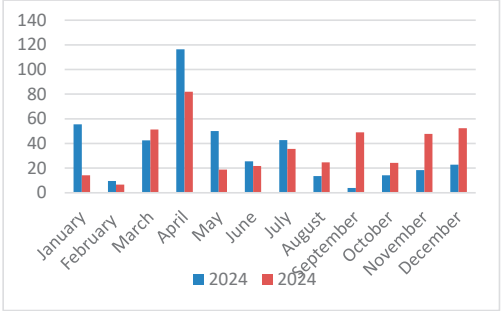


Figure 2. Rainfall in experimental field (2023-2024)

The field experiment was based on the method of subdivided plots into 3 replications, having two experimental factors (bifactorial experiment), respectively:
- Factor A: Tillage, with five variants:
  - a1. Plowing performed with a 4-furrow reversible plough at depth of 25 cm + 2

passes with a disc harrow (Control variant);
  - a2. Scarifying with Artiglio Scarifier at a depth of 35 cm + 2 passes with a disc harrow;
  - a3. Cultivation with Gruber Tiger cultivator at a depth of 25 cm;
  - a4. Cultivation with Gruber Tiger cultivator at a depth of 15 cm;
  - a5. Disc harrow with Horsh Joker Disc at a depth of 15 cm (2 passes).
- Factor B; Nitrogen rate, with four variants:
  - b1. N0 - unfertilized (Control variant);
  - b2. 80 kg/ha;
  - b3. 120 kg/ha;
  - b4. 160 kg/ha.

Each experimental variant had 120 m^2, resulting from 20 m length and 6 m width.

The tillage according to the classic system (plowing and scarifying) took place in the previous fall, and the minimum tillage (Gruber Tiger 15 cm, Gruber Tiger 25 cm, Disc harrow (2 passed) were performed in the spring.

In all experimental variants, except for Control variant, before seedbed preparation there was applied the complex fertilizer 16:16:16 in a rate of 250 kg/ha, assuring 40 kg/ha as active substance of N, P_2O_5 and K_2O. In May (1-5 of May), the nitrogen rate according to the experimental variant was assured by the second fertilization applying the liquid fertilizer UAN (Urea Ammonium Nitrate Solution) containing 32% nitrogen. Thus, in 2023, on 19th of May, and two weeks early in 2024, on 5th of May, there was applied 125 l/ha of UAN for the variant b2, 250 l/ha of UAN for the variant b3, and 375 l/ha of UAN for the variant b4.

The previous plant was maize, and the maize hybrid in the field experiment was P9911.

One day before sowing, the seedbed preparation was carried out with a combinatory. The sowing was carried out on 3rd of May in 2023 and 2 weeks early in 2024, respectively on 20th of April, because the weather was warm and dry. The sowing was carried out with a John Deer tractor and a Gaspardo Maestro seeder with 8 rows, at a depth of 5-6 cm, a distance between rows of 70 cm and a sowing density of 66,000 germinating grains/ha.

Herbicide treatments were applied for controlling annual and perennial weeds, such as: Roundup Extra (360 g/l Glyphosate) herbicide applied pre-emergent at a rate of 1.5 l/ha; Adengo 465 SC (225 g/l Isoxaflutole + 90 g/l Thiencarbazone-methyl + 150 g/l Cyprosulfamide as safener) herbicide applied early post-emergent (on middle of May) at a rate of 0.4 l/ha; Principal Plus (92 g/kg Nicosulfuron + 550 g/kg Dicamba + 23 g/kg Rimsulfuron) herbicide applied post-emergent (at beginning of June) at a rate of 0.4 kg/ha against annual grasses and perennial and annual broadleaf weeds.

For controlling the adults of *Diabrotica virgifera* Leconte (western maize rootworm), Rexaris 25 WG (25 g/kg Lambda-Cyhalothrin) insecticide was applied at a rate of 0.25 kg/ha.

For controlling *Tanymecus dilaticollis* Gyll. (maize leaf weevil) and *Agriotes* spp. (wireworms), Krima 20 SG (200 g/kg Acetamiprid) insecticide was applied after plant emergence, at a rate of 0.1 kg/ha.

The insecticide Coragen (200 g/l Chlorantraniliprole), at a rate of 0.2 l/ha, was applied at the time of panicle emergence, against *Helicoverpa armigera* (Hübner) (corn earworm).

Harvesting was carried out with the New Holland CR9090 combine on 15th of September in 2023 and on 1st of September in 2024.

For each variant, the following determinations were performed at harvest: cob length (cm); number of grain rows per cob; number of grains per row; number of grains per cob; cob weight (g); grain weight per cob (g); grain yield (kg/ha).

RESULTS AND DISCUSSIONS

In the year 2023, which was more favourable from a climatic point of view compared to 2024, all the yielding elements of the maize plant registered the smallest values in the case of variant with 0 kg/ha nitrogen and tillage method plowing, except the no. of grain rows per cob which had the smallest values in the case of variant with tillage method disc harrow (Table 1).

The highest values of the yielding elements of the maize plant were registered in 2023 in the case of tillage method with disc harrow and nitrogen fertilization with the highest rate, respectively 160 kg/ha, except the case of no.

of grain rows per cob were the highest values was registered at the nitrogen rate of 120 kg/ha. Also very close to maximum values of the cob weight and grain weight per cob were registered in the case of variant of tillage with Gruber Tiger at 25 cm depth, and again fertilized with the highest nitrogen rate of 160 kg/ha. The high values in the case of this tillage method have led to the highest value of the grain yield of 11,494 kg/ha. The smallest grain yield was registered also in the case of the tillage method with Gruber Tiger at 25 cm depth, but with no nitrogen fertilization (0 kg/ha of nitrogen).

Compared to plowing variant with 0 kg/ha of nitrogen in 2023, all the other tillage methods determined negative differences in the case of no nitrogen fertilization, while adding nitrogen determined positive differences regardless of tillage method, the highest grain yields being at the highest nitrogen rate (160 kg/ha). But, increasing the nitrogen rate (80, 120, and 160 kg/ha) determined the highest yield increase in the case of tillage with Gruber Tiger at 25 cm depth, while the smallest yield increase was registered in the case of tillage method with Gruber Tiger at 15 cm depth (Table 1).

In the year 2024, which was less favourable from a climatic point of view compared to 2023, as in the case of the year 2023, all the yielding elements of the maize plant registered the smallest values in the case of variant with 0 kg/ha nitrogen but at tillage method scarifying, except the cob length were the smallest value was in the case of tillage method plowing and except the no. of grain rows per cob were the smallest value was in the case of tillage method disc harrow (Table 2).

The highest values of the yielding elements of the maize plant were registered in 2024 at the highest rate of nitrogen of 160 kg/ha and tillage method of Gruber Tiger at 25 cm depth in the case of no. of grains per cob and grain weight per cob, the tillage method of Gruber Tiger at 15 cm depth in the case of cob length and no. of grains per row.

Some of the yielding elements registered the highest values at the nitrogen rate of 120 kg/ha and the tillage method Gruber Tiger at 25 cm depth in the case of cob weight, and the tillage method disc harrow in the case of no. of grain rows per cob.

The highest value of the grain yield of 3972 kg/ha was registered in the variant with tillage method Gruber Tiger at 15 cm depth and nitrogen rate of 160 kg/ha. The smallest value of the grain yield was registered in the variant with tillage method with scarifying and no nitrogen fertilization (0 kg/ha of nitrogen).

Compared to plowing variant with 0 kg/ha of nitrogen in 2024, only the variant with tillage method scarifying determined a negative difference in the case of no nitrogen fertilization. Adding nitrogen determined positive differences regardless of tillage method, the highest grain yields being at the highest nitrogen rate (160 kg/ha). But, increasing the nitrogen rate (80, 120, and 160 kg/ha) determined the highest yield increase in the case of tillage with Gruber Tiger at 15 cm depth, as well as with Gruber Tiger at 25 cm depth (Table 2).

As average values for tillage methods in 2023, generally the variant with tillage method disk harrow gave the highest values for the yielding elements except the case of cob weight which was the highest at tillage method Gruber Tiger at 25 cm depth, the variant which gave also the highest grain yield, this having a good effect also on plants population and on the no. of cobs per plant, respectively per hectare (Table 3).

As average values for tillage methods in 2024, the variant with tillage method Gruber Tiger at 25 cm depth gave the highest values for the yielding elements, except for no. of grain rows per cob, as well as the highest value for the grain yield (Table 4).

As average values for the nitrogen rate, in both experimental years (2023 and 2024), the highest values for yielding elements and grain yield were obtained in the case of variant fertilized with 160 kg/ha of nitrogen (Tables 5 and 6). It can be highlighted that generally the nitrogen rate had a beneficial effect on all the analysed parameters, but especially at the highest rate.

Comparatively assessing the results obtained during the two experimental years, for the less favourable year 2024, it can be seen that the values of all analysed parameters were lower compared to year 2023.

Table 1. Yielding elements of maize plants and grain yield at different tillage methods and nitrogen rates, in 2023

Tillage methods	Yielding elements							Grain yield (kg/ha)		
	Nitrogen rate (kg/ha)	Cob length (cm)	No. of grain rows per cob	No. of grains per row	No. of grains per cob	Cob weight (g)	Grain weight per cob (g)	Value	*Dif. from N0*	*Dif. from plowing and N0*
Plowing (25 cm + 2 disc harrows) (Control)	N0	15.1	16.6	24.4	404.1	108.8	93.1	8871	*0*	*0*
	N80	17.1	17.3	29.6	511.4	149.8	129.6	9105	*234*	*234*
	N120	17.3	17.2	30.9	532.0	155.5	133.3	9218	*347*	*347*
	N160	17.3	17.3	30.4	525.0	161.0	139.8	9488	*617*	*617*
Scarifying (35 cm + 2 disc harrows)	N0	17.1	17.1	30.3	518.6	164.6	140.7	8625	*0*	*-246*
	N80	17.6	16.8	32.9	551.2	186.7	160.2	9153	*528*	*282*
	N120	17.5	17.4	32.3	560.8	180.3	156.6	9209	*584*	*338*
	N160	18.0	17.2	33.6	578.4	189.8	165.2	9935	*1310*	*1064*
Gruber Tiger (25 cm)	N0	17.0	17.0	30.1	511.4	157.8	135.8	8397	*0*	*-474*
	N80	18.0	17.1	33.1	565.4	185.9	153.8	9559	*1162*	*688*
	N120	18.1	17.4	33.4	580.3	194.8	168.2	10618	*2221*	*1747*
	N160	18.6	17.3	34.5	596.3	207.3	177.5	11494	*3097*	*2623*
Gruber Tiger (15 cm)	N0	18.0	16.9	31.4	531.6	162.7	139.1	8822	*0*	*-49*
	N80	17.6	17.0	32.2	546.6	172.0	147.0	9037	*215*	*166*
	N120	17.9	17.1	32.3	552.6	179.8	153.3	9107	*285*	*236*
	N160	18.3	17.0	34.7	591.3	192.9	166.0	9349	*527*	*478*
Disc harrow (15 cm x 2 passes)	N0	17.4	16.3	32.2	524.0	178.3	150.6	8560	*0*	*-311*
	N80	17.6	17.5	32.2	563.5	185.7	160.2	8840	*280*	*-31*
	N120	17.9	17.9	33.1	591.1	184.3	158.8	9054	*494*	*183*
	N160	19.2	17.5	36.1	630.7	206.0	177.6	9253	*693*	*382*

Table 2. Yielding elements of maize plants and grain yield at different tillage methods and nitrogen rates, in 2024

Tillage methods	Yielding elements							Grain yield (kg/ha)		
	Nitrogen rate (kg/ha)	Cob length (cm)	No. of grain rows per cob	No. of grains per row	No. of grains per cob	Cob weight (g)	Grain weight per cob (g)	Value	*Dif. from N0*	*Dif. from plowing and N0*
Plowing (25 cm + 2 disc harrows) (Control)	N0	12.4	12.4	20.1	249.7	121.6	99.8	1630	0	0
	N80	13.3	13.2	19.0	250.4	164.0	159.9	2111	481	481
	N120	14.8	13.7	26.2	358.1	168.3	141.6	2081	451	451
	N160	15.3	13.9	23.3	324.4	171.8	160.8	2966	1336	1336
Scarifying (35 cm + 2 disc harrows)	N0	13.7	13.9	14.7	203.9	109.2	91.0	1585	0	-45
	N80	15.1	12.7	16.1	204.1	147.8	122.1	2400	815	770
	N120	15.3	13.3	22.5	299.8	148.8	123.9	2429	844	799
	N160	15.7	13.3	23.3	310.9	157.7	130.5	2667	1082	1037
Gruber Tiger (25 cm)	N0	14.4	13.3	22.6	301.7	137.8	113.3	1891	0	261
	N80	15.0	14.1	25.2	355.1	206.5	169.8	2918	1027	1288
	N120	15.5	15.4	27.0	415.0	208.2	172.8	3253	1362	1623
	N160	15.8	15.6	28.6	445.5	207.3	177.5	3735	1844	2105
Gruber Tiger (15 cm)	N0	13.0	12.6	16.7	210.5	140.7	114.4	1799	0	169
	N80	13.6	13.0	26.8	347.7	172.0	140.6	2358	559	728
	N120	14.9	13.8	28.1	387.1	179.8	153.3	3143	1344	1513
	N160	15.9	14.4	29.4	422.6	192.9	166.0	3972	2173	2342
Disc harrow (15 cm x 2 passes)	N0	12.6	12.2	21.4	261.4	169.9	139.0	1679	0	49
	N80	13.4	17.8	15.3	272.0	175.7	143.9	2251	572	621
	N120	14.6	17.9	19.8	353.8	184.3	151.8	2622	943	992
	N160	15.1	17.5	20.6	359.9	206.0	172.6	3235	1556	1605

Table 3. Yielding elements of maize plants and grain yield at different tillage methods in 2023

Yielding elements and grain yield	Tillage method				
	Plowing (25 cm + 2 disc harrows) (Control)	Scarifying (35 cm + 2 disc harrows)	Gruber Tiger (25 cm)	Gruber Tiger (15 cm)	Disc harrow (15 cm x 2 passes)
Cob length (cm)	16.7	17.6	17.9	17.9	18.0
No. of grain rows per cob	17.1	17.1	17.2	17.0	17.3
No. of grains per row	28.8	32.3	32.8	32.6	33.4
No. of grains per cob	493.1	552.3	563.3	555.5	577.3
Cob weight (g)	143.8	180.3	186.4	176.8	188.6
Grain weight per cob (g)	123.9	155.7	158.8	151.3	161.8
Grain yield (kg/ha)	9171	9231	10017	9079	8927

Table 4. Yielding elements of maize plants and grain yield at different tillage methods in 2024

Yielding elements and grain yield	Tillage method				
	Plowing (25 cm + 2 disc harrows) (Control)	Scarifying (35 cm + 2 disc harrows)	Gruber Tiger (25 cm)	Gruber Tiger (15 cm)	Disc harrow (15 cm x 2 passes)
Cob length (cm)	14.0	14.9	15.2	14.4	13.9
No. of grain rows per cob	13.3	13.3	14.6	13.4	16.3
No. of grains per row	22.2	19.1	25.8	25.2	19.3
No. of grains per cob	295.7	254.7	379.3	342.0	311.8
Cob weight (g)	156.4	140.6	189.9	171.3	184.0
Grain weight per cob (g)	140.5	116.9	158.4	143.6	151.8
Grain yield (kg/ha)	2197	2271	2950	2818	2447

Table 5. Yielding elements of maize plants and grain yield at different nitrogen rates in 2023

Yielding elements and grain yield	Nitrogen rate (kg/ha)			
	N0 - Unfertilised (Control)	N80	N120	N160
Cob length (cm)	16.9	17.6	17.8	18.3
No. of grain rows per cob	16.8	17.1	17.4	17.3
No. of grains per row	29.7	32.2	32.4	33.9
No. of grains per cob	498.0	547.6	563.4	584.3
Cob weight (g)	154.4	176.0	178.9	191.4
Grain weight per cob (g)	131.9	150.2	154.0	165.2
Grain yield (kg/ha)	8655	9139	9441	9904

Table 6. Yielding elements of maize plants and grain yield at different nitrogen rates in 2024

Yielding elements and grain yield	Nitrogen rate (kg/ha)			
	N0 – Unfertilised (Control)	N80	N120	N160
Cob length (cm)	13.2	14.1	15.0	15.6
No. of grain rows per cob	12.9	14.1	14.8	14.9
No. of grains per row	19.1	20.5	24.7	25.0
No. of grains per cob	245.4	285.9	362.8	372.7
Cob weight (g)	135.8	173.2	177.9	187.1
Grain weight per cob (g)	111.5	147.3	148.7	161.5
Grain yield (kg/ha)	1717	2408	2706	3315

CONCLUSIONS

The results obtained under the experimental conditions from the Danube Meadow in South Romania show the positive effect of nitrogen fertilization on maize yielding elements and grain yield, especially at the highest rate regardless of the tillage method or climatic conditions of the year.

The variant of conventional tillage with plowing followed by 2 passes with a disc harrow gave the smallest grain yields regardless of the climatic conditions of the year.

The highest average grain yields were obtained in the case of tillage variant with Gruber Tiger at 25 cm depth, especially in better climatic conditions associated with high nitrogen rate (160 kg/ha). In less favourable climatic conditions, good maize yields were obtained also in the case of tillage with Gruber Tiger at 15 cm depth, also associated with high nitrogen rate.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

THE IMPACT OF PEDOCLIMATIC CONDITIONS ON THE PRODUCTION OF ESSENTIAL OIL AND LAVENDER HYDROLATE

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Abstract

Lavender is a particularly valuable plant, having multiple uses and economic benefits (oil production, derived products, tourism), medicinal (aromatherapy, therapeutic properties) and gastronomic. Its cultivation combines economic and ecological benefits, contributing to the creation of sustainable agricultural practices. The present study refers to the amount of lavender essential oil and hydrolate, that can be extracted depending on the year of cultivation and the specific pedoclimatic conditions of three cultivation areas: Deta (Timiș County), Mailat and Vinga (Arad County). The climatic conditions of the analyzed period had a positive impact on the amount of volatile oil and lavender hydrolate. The amount of lavender oil, ranged from 10.7 liters/hectare at Deta and 13 liters/hectare at Vinga, in the second year and between 31 liters/hectare at Deta and 35 liters/hectare at Mailat, in the third year. Regarding the amount of lavender hydrolate, was between 111 liters/hectare in Deta and 129 liters/hectare in Vinga, in the second year and between 310 liters/hectare in Deta and 392 liters/hectare in Mailat, in the third year.

Key words*: essential oil, hydrolate, Lavandula sp., sustainable agriculture.*

INTRODUCTION

Lavender (*Lavandula* sp.) is an aromatic shrub native to the Mediterranean region, used in many fields due to its high content of essential oils. It is especially appreciated in the cosmetics and pharmaceutical industries, but also in food and landscaping (Mac & Harris, 2002; Kara & Baydar, 2013; Giray, 2018).

Of the 39 species belonging to the genus *Lavandula*, only 17 have been studied in detail for the chemical composition of volatile oils. *Lavandula angustifolia* is recognized as the most valuable species for essential oil production due to its chemical composition and extensive applicability in the pharmaceutical, medical, and cosmetic fields (Yukes & Balick, 2010; Salehiá et al., 2018; Pokajewicz et al., 2021; Butta et al., 2024).

Chemically, lavender oil contains mainly linalool (20-45%) and linalyl acetate (25-47%), compounds responsible for its characteristic aroma and therapeutic benefits. In addition to these, other compounds are also present in lower concentrations, such as eucalyptol (≤2.5%) and camphor (≤1.2%), the latter being associated with lower oil quality (Ceaușescu, 1988; Lis-Balchin, 2002; Wells et al., 2018; Tăbărașu et al., 2023).

Lavender essential oil is mainly used for cosmetic and therapeutic purposes, being recognized for its antiseptic, soothing and sedative effects, especially in the treatment of insomnia and stress (Sönmez et al., 2018; Zhu et al., 2018). According to the standards of the European Pharmacopoeia, the use of oil obtained by distillation of dried flowers (13 ml/kg) and that extracted from the upper stems is allowed in the pharmaceutical industry (Mykhailenko et al., 2022).

The quality and chemical composition of lavender oil are influenced by several factors, including the species and variety used, environmental conditions, cultivation technology, and distillation methods. For example, steam distillation favors a higher concentration of alpha-terpineol, linalool, and linalyl acetate, compounds that contribute to its therapeutic and commercial properties (Parkash & Singh, 2013). From a taxonomic point of view, the genus groups 39 species, classified in three subgenera (*Lavandula, Fabricia, Sabaudia*) and eight sections (*Lavandula, Dentatae, Stoechas, Pterostoechas, Subnudae*,

Chaetostachys, Hasikenses and Sabaudia). The number of taxa is much higher (up to 90), if we also consider taxa, subspecies and hybrids (Pokajewicz et al, 2023; Haban et al., 2023). Botanically, lavender species marketed for essential oil are part of the section *Lavandula* and include *Lavandula angustifolia*, *Lavandula latifolia* and *Lavandula x intermedia*. In contrast, species belonging to the section *Stoechas* and *Pterostaechas* are mostly used for ornamental purposes (Yukes & Balick, 2010; Salehi et al., 2018; Batiha et al., 2023).

The components of lavender essential oil are grouped into two main categories: terpene hydrocarbons (monoterpenes, sesquiterpenes, diterpenes) and oxygenated compounds derived from them (alcohols, aldehydes, esters, ketones, phenols and oxides). Of the monoterpenes, the most valuable are linalool and linalyl acetate, which contribute to the characteristic aroma and superior quality of the essential oil (Urwin, 2009; Rus et al., 2016; Bialon et al., 2019; Batiha et al., 2023). Other minority compounds include cineole, borneol, β-pinene, terpineol, and camphor, the latter of which is considered a factor in quality reduction (Baser, 1993; Bialon et al., 2019; Masyita et al., 2022; Beicu et al., 2022).

In addition to the essential oil, another valuable by-product is lavender water, obtained as a by-product in the distillation process. It has antifungal and antibacterial properties, being used especially in the cosmetic industry, in the formulation of face and body lotions (Kunicka et al., 2015; Imbrea et al., 2016; Bălășoiu et al., 2024).

MATERIALS AND METHODS

The research was carried out on three lavender plantations from different localities in the Banat area, namely Deta (Timiș County), Vinga and Mailat (Arad County). The objective of the research is the behavior of a variety of *Lavandula angustifolia* in the crop in terms of the level of lavender volatile oil and lavender water productions, obtained in different years. The year 2023 represents the first year of study and is the 2nd year after planting, and the year 2024 represents the 2nd year of study, namely the 3rd year after planting. Harvesting was done starting with the second year after planting to allow the development of the root system of the plant, therefore it was not distilled in order to obtain lavender oil and water.

To determine the amount of lavender/plant flowers (inflorescence), 25 individuals from each location were sampled and only the tips of the stem were cut 3-5 cm below the inflorescence. The quantity of flowers/individual was weighed, the number of cuttings per hectare was taken into account and the average quantity resulting from the determinations on the 25 individuals was multiplied by the number of cuttings/hectare. Knowing the quantity of flowers per hectare for each site, three distillations were carried out for each site for a sample of 10 kg of fresh flowers and the extraction yield for each 10 kg of flowers was calculated. To determine the amount of lavender oil and water per hectare, the amount of oil and water obtained using the formula was mathematically calculated:

$$\text{Oil production } (ha) = \frac{\text{U10}}{10} x\ Fha,$$

where: U10 is the average amount of oil obtained from 10 kg of flowers and Fha is the amount of flowers per hectare.

The same formula was used to determine the amount of lavender water per hectare:

$$\text{Lavender water production } (ha) = \frac{\text{U10}}{10} x\ Fha,$$

where: U10 is the average amount of lavender water obtained from 10 kg of flowers and Fha is the amount of flowers per hectare.

RESULTS AND DISCUSSIONS

The synthesis of the results obtained according to the crop year and the quantitative variations of the oil per hectare are presented in Table 1. From the analysis of the data, it can be seen that, in the crop year 2023 (A1), the production of essential oil was 12.08 l/ha, and in 2024 (A2), this value increased to 33.33 l/ha, representing an increase of 270% compared to the previous year. The positive difference of 21.27 l/ha exceeds the DI 0.1% (20.51), which means that this increase is very statistically significant. This increase is explained by the fact that lavender being a perennial crop, in the first years, the yield is lower, but reaches an optimal peak starting with the 3rd or 4th year of cultivation (Lis-Balchin, 2002).

Table 1. Amount of oil/ha by crop year

Year of culture	Oil/ha (l)	%	Difference (l)	Significance
A1 - 2023	12.06	100		
A2 - 2024	33.33	276	21.27	***
DL 5% = 2.79; DL 1% = 6.44; DL 0.1% = 20.51				

Table 2 shows the results regarding the amount of oil/ha depending on the cultivation location. The data is organized across three different locations: Deta, Vinga, and Mailat. From the analysis of the results, we can observed, that the amount of oil depending on the location, had values of 20.85 l/ha at Deta, 23.1 l/ha at Vinga, which represents an increase of 10% compared to Deta and 23.75 l/ha at Mailat, which represents an increase of 14% compared to Deta (considered a control).

Analyzing the results obtained, we can say that there is a variability of the oil content depending on the cultivation location. The highest values of oil are in Mailat (23.75 l/ha), and the lowest in Deta (20.85 l/ha). These results suggest that location significantly influences oil accumulation, which may have implications for agriculture and plant nutrition.

Table 2. Amount of oil/ha depending on the cultivation location

Locality	Oil/ha (l)	%	Difference (l)	Significance
b1 - Deta	20.85	100		
b2 - Vinga	23.50	113	2.65	Ns
b3 - Mailat	23.75	114	2.9	Ns
Dl 5% = 3.42; Dl 1%=7.89; Dl 0.1% = 25.11				

TEST DUNCAN - factor B, DL5% = 3.42

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Mean	1 =	20.85	A		
Mean	2 =	23.50	A		
Mean	3 =	23.75	A		

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Mean	3 =	23.75	A		
Mean	2 =	23.50	A		
Mean	1 =	20.85	A		

Mean 1 – b1 – Deta
Mean 2 – b2 – Vinga
Mean 3 – b3 – Mailat

Although there is an apparent increase in oil production/ha in Mailat and Vinga compared to Deta, these differences are not statistically significant.

The highest value 23.75 was obtained in the Mailat location - class A, and the lowest 20.85, in the Deta location - class A. Between the values obtained at each location, there are no statistically assured differences, they are part of the same homogeneity class - class A.

The differences observed between the locations could be caused by natural variations or external factors, but they are not large enough to be considered statistically relevant. We can also state that although there is variation between locations, it is not large enough to justify major changes in cultivation technologies

The percentage contribution of different factors (crop year, location and error), to the variation in the probable oil yield/ha is shown in Figure 1.

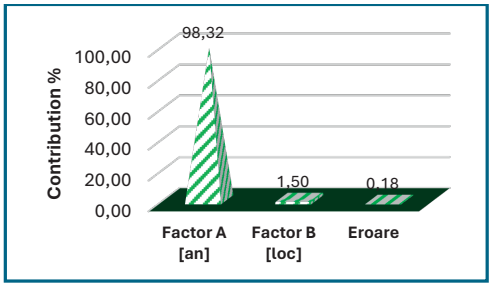


Figure 1. Contribution of factors A [crop year], B [crop locality] to lavender oil content

Factor A (crop year) contributes the most with 98.32%, this suggests that the age of the lavender crop plays a dominant role in determining the yield.

The B factor (location) has a minor contribution of 1.50%, the influence of location indicates that although location may have some effect, it is not the main determinant of yield variation.

The error variation is negligible at 0.18%, the unexplained variation (random errors or experimental uncertainties), is extremely low. This implies that the study design is robust and the results are very reliable.

The results of the cluster analysis of lavender oil content by crop year and location are shown in Figure 2.

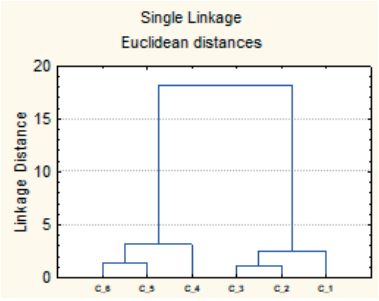


Figure 2. Cluster analysis of lavender oil content by crop year and location

This dendrogram suggests that the dataset contains well-defined groups, and the Single Linkage method produces a "chain" effect in

which closer objects are grouped quickly, but large groups are joined at large distances. This can be useful if the goal is to discover elongated structures in the data.

Analyzing the growing areas, the results presented in the dendrogram indicate similar plant growth conditions (C_1 and C_2 are very close, suggesting that they could be two varieties related or having similar agronomic characteristics). The results of the impact of the lavender crop year on the amount of hydrolate/ha are presented in Table 3. From the analysis of the data, a significant difference can be observed between 2023 and 2024 in terms of the amount of hydrolate obtained per hectare.

If in 2023, the hydrolate production/ha was 123 l, considered the reference base (100%), in 2024, the production increased considerably to 361 l/ha, which represents an increase of 238 l/ha compared to the previous year, distinctly statistically significant difference. These results prove that lavender being a perennial crop, the amount of hydrolate increases as it advances in vegetation.

Table 3. Quantity of hydrolate/ha by crop year

Year of culture	Hydrolate/ ha (l)	%	Difference (l)	Significance
A1 - 2023	123	100		
A2 - 2024	361	293	238	**
DL 5% = 85.45; DL 1%= 197.09; DL 0.1% = 627.31				

With reference to the influence of location on hydrolate production (Table 4), the results show that these significant variations between the three analyzed locations: Deta, Vinga and Mailat.

Table 4. Amount of hydrolate/ha depending on the cultivation location

Locality	Hydrolate/ha (l)	%	Difference (l)	Significance
b1 - Deta	211	100		
b2 - Vinga	255	121	44	Ns
b3 - Mailat	260	123	49	Ns
DL 5% = 104.65; DL 1%=241.38; DL 0.1% = 768.29				

The lowest amount of hydrolate/ha was recorded in the experimental field from Deta, 211 l/ha, followed by Vinga with a production of 255 l/ha, which means a difference of 44 l/ha compared to Deta and Mailat with the highest production, 260 l/ha, an increase of 49 l/ha compared to Deta. Even if there are differences between locations, these differences are insignificant.

TEST DUNCAN - factor B, DL5% = 104.6

Date originale				Date sorate			
Mean	1 =	210.5	A	Mean	3 =	260.0	A
Mean	2 =	254.5	A	Mean	2 =	254.5	A
Mean	3 =	260.0	A	Mean	1 =	210.5	A

Mean 1 - b1 - Deta
Mean 2 - b2 - Vinga
Mean 3 - b3 - Mailat

The highest value, 260 liters, was obtained in the Mailat location - class A, and the lowest 210.5 liters, in the conventional Deta location - class A. Between the values obtained at each location there are no statistically assured differences, they are part of the same homogeneity class - class A.

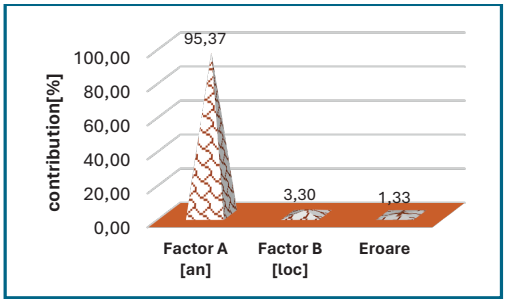


Figure 3. Contribution of factors A [crop year], B [crop locality] to lavender hydrolate content

Analyzing the results obtained, it can be seen that Factor A (year of cultivation) has the greatest influence (95.37%), which means that the differences between locations were not significant or have a much lower impact on the parameter studied, and the Experimental Error (1.33%) is very small, which suggests a high precision of the experiment and a good reproducibility of the results (Figure 3).

The results of the cluster analysis for lavender hydrolate content by crop year and location are shown in Figure 4.

Analyzing the data, it is observed that there are two large groups, united at a very large distance (~160-180), indicating major differences between them.

The clusters C_5 and C_4 are the closest, suggesting a high similarity in hydrolate content between these two samples. Another smaller group is formed between C_3 and C_2, and C_1 is more isolated and different from the others

In conclusion, we can also say from this analysis that in terms of hydrolate content, the age of the plants has a greater influence than the location.

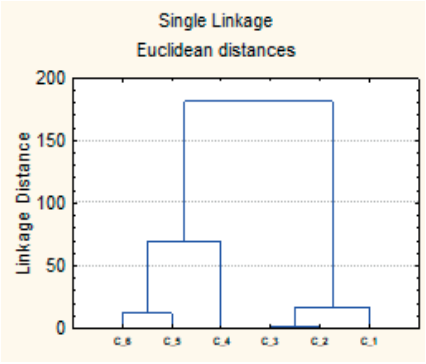


Figure 4. Cluster analysis of lavender hydrolate content by crop year and location

The correlation between the amount of flowers and lavender oil is shown in Figure 5.

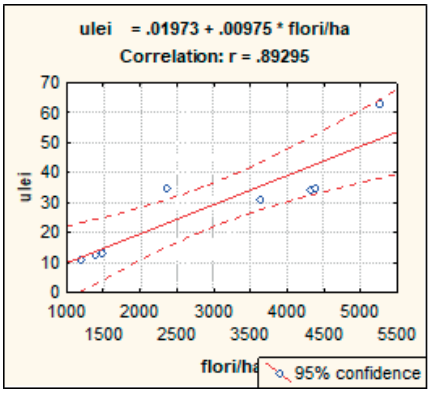


Figure 5. Correlation between the amount of flowers and lavender oil

The linear regression equation indicates that there is a positive linear relationship between the number of flowers per hectare and the amount of oil obtained. In other words, as the density of the flowers increases, so does the amount of oil extracted. According to the correlation coefficient (R=0.89295), the correlation between flowers and oil is very strong, because r is close to 1. This means that the variables are closely related: an increase in the number of flowers per hectare causes a significant increase in the amount of oil. The regression model suggests that, on average, for each increase of 1000 flowers/ha, the amount of oil increases by about 9.75 units. This is valuable information for optimizing the production of lavender essential oil.

The correlation between the amount of flowers and lavender hydrolate shown in Figure 6, indicates that there is a positive linear relationship between the number of flowers per hectare and the amount of hydrolate obtained.

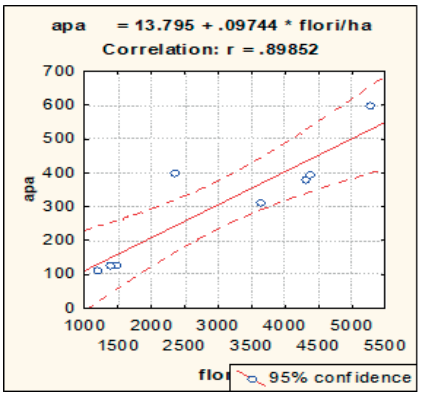


Figure 6. Correlation between the amount of flowers and lavender hydrolate

The linear regression equation indicates that there is a positive linear relationship between the number of flowers per hectare and the amount of hydrolate obtained. More precisely, each growth with 1000 flowers/ha determines an average growth of about 97.44 hydrolate units.
Figure 7 shows the results of the correlation between the amount of herba and lavender oil.

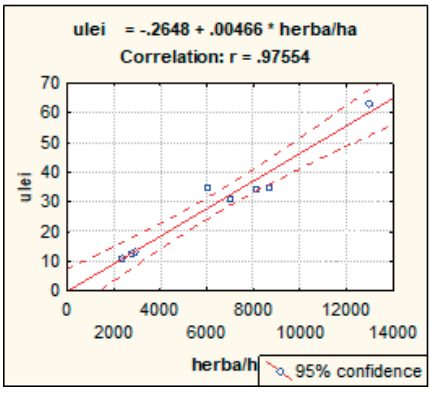


Figure 7. Correlation between the amount of herb and lavender oil

The linear regression equation indicates a positive linear relationship between the amount of herba per hectare and the amount of lavender oil obtained.

For every 1000 kg of grass/ha, the amount of oil increases by an average of 4.66 units.
The correlation coefficient (r=0.97554) is very close to 1, which means that the variations in the amount of grass/ha almost entirely explain the variation in the amount of oil obtained.
Correlation between the amount of herb and lavender hydrolate (Figure 8). The linear regression equation indicates a positive linear relationship between the amount of grass harvested per hectare and the amount of lavender hydrolate obtained. Each 1000 kg of grass/ha causes an average growth of 45.8 units of hydrolate. The correlation is very strong, suggesting that more harvested grass leads to a significant increase in the amount of hydrolate.

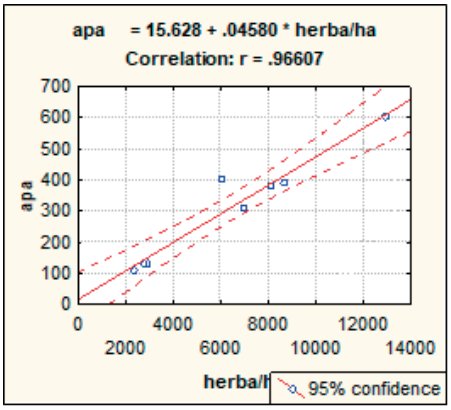


Figure 8. Correlation between the amount of herb and lavender hydrolate

Correlation between the amount of oil and lavender hydrolate (Figure 9).

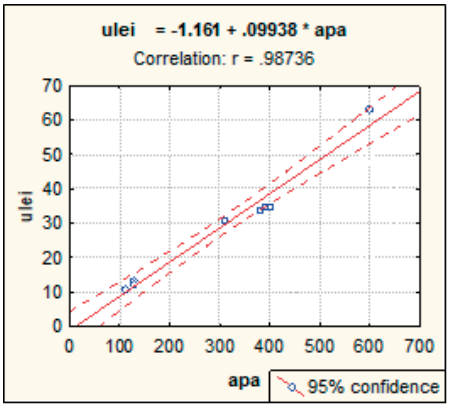


Figure 9. Correlation between the amount of oil and lavender hydrolate

The linear regression equation indicates a positive linear relationship between the amount of hydrolate (lavender water) and the amount of oil obtained.
Every 100 units of hydrolate results in an average increase of 9,938 units of oil.
The correlation coefficient value (r=0.98736) indicates the existence of an extremely strong correlation between the amount of hydrolate and lavender oil.
The 95% confidence interval indicates that the reliability of the model is very high, which means that this relationship can be used to optimize the distillation process.

CONCLUSIONS

The production of lavender oil and hydrolate varies depending on the year of cultivation, in the first two years after planting, lavender has a lower yield of essential oil and hydrolate, as the plants are still developing. From the 3rd year of cultivation, the plants reach maturity and the production of flowers and grass increases significantly, also influencing the production of oil and hydrolate.
The cluster analysis confirms that the year of cultivation is the main influencing factor on the oil content and the lavender hydrolate content, and the location plays a secondary role.
There is also a strong positive relationship between the number of lavender flowers per hectare, the amount of herba/ha and the amount of oil and hydrolate obtained.
Farmers and producers of lavender oil and hydrolate can use this model to estimate hydrolate production based on the amount of flowers and grass harvested per hectare.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

INFLUENCE OF CLIMATIC CONDITIONS ON POTATO TUBERS QUALITY

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Abstract

The classification of potato tubers into usage types (human consumption, industrial processing, or animal feeding), in addition to factors such as variety and fertilization, can be influenced by the crop climatic conditions. Over three years of non-irrigated potato crop in Țara Bârsei - Brașov (2022-2024), the varying climatic conditions affected the culinary and technological quality parameters of potato tubers. The dry matter content, consistency, mealiness, moisture of the potato tubers, and the structure of starch granules were influenced by air temperature and precipitation levels during the growing season, as these conditions impact growth, metabolism and the accumulation of reserve substances in the tubers. The variation in these culinary quality traits of potato tubers was analyzed across the production of 12 potato varieties. The varieties were classified into the following usage types: A, AB, B, and BC. Over the three years of observations, the varieties Azaria and Ervant recorded the highest and respectively the lowest accumulation of dry matter. Careful management of environmental factors, combined with the use of well-adapted varieties, can optimize the potato quality for various usage types.

Key words*: potato, climatic condition, tubers quality.*

INTRODUCTION

Several factors influence the quality of potato tubers, affecting their technological quality, texture, taste, cooking properties, and overall market value. These factors can be categorized into environmental conditions, agricultural practices, genetic traits, and post-harvest handling. Drought stress is a major constraint on potato production, as adequate water availability is essential for achieving high yields and maintaining tuber quality (Rudack et al., 2017). Research on the influence of climatic conditions on the quality parameters of potato tubers showed that drought stress caused by insufficient precipitation has been found to limit potato plant growth and development by reducing photosynthetic activities in leaves, resulting in decreased tuber yield and tuber dry mass (Nasir and Toth, 2022). Rymuza et al. (2015) showed that starch content in tubers of medium-early potato cultivars depends more on precipitation than on thermal conditions. Soil water stress restrict potato plant growth and development, reduce amylase activity, thereby reduce tuber starch accumulation (Yong Zhen Ma et al., 2024). Short period of water deficit especially in the vegetative and tuberization phases, have negative impacts while short period of water excess have lightly positive impacts on potato vegetative development and tuber yield and quality (Wagg et al., 2021).

Water shortage during the growing season contribute to a deterioration of the potato culinary characteristics, while, on the contrary, excess water contribute to their improvement (Bienia et al., 2020).

Starch content is an important quality indicator of potatoes (Wang et al., 2021). The starch content of potato tubers is closely related to their crushing on boiling, consistency, mealiness, and moisture content. Potatoes with high starch content tend to disintegrate or become soft and fluffy when boiled, whereas those with lower starch content remain firm and intact. High-starch potatoes have a drier, mealy

texture, whereas low-starch varieties are smoother and more cohesive. High-starch potatoes have lower moisture content, making them drier, while low-starch potatoes have higher moisture content, making them more waxy and dense.
The main objective of this study was to identify potato varieties with stability of the technological and culinary tubers quality characteristics under the variability of hydrothermal conditions.

MATERIALS AND METHODS

Research on the tuber quality of ten mid-early potato varieties (Brașovia, Castrum, Marvis, Sarmis, Sevastia, Azaria, Darilena, Asinaria, Cezarina, Ervant) and two mid-late potato varieties (Cosiana, Foresta) cultivated in the experimental field of National Institute for Research and Development for Potato and Sugar Beet (NIRDPSB) Brașov, was carried out from 2022 to 2024.
These twelve potato varieties developed through the breeding program of NIRDPSB Brașov, were monitored regarding the stability of the technological and culinary tubers quality characteristics under the influence of variability of climatic condition, air temperature (°C) and amount of rainfall (mm).
All varieties were grown under the same agroclimatic conditions over the three years of study, in a non-irrigated crop, following the same cultivation technology. Potato planting took place on April 9 in 2022, April 28 in 2023, and April 10 in 2024, while harvesting was carried out on September 19 in 2022, September 13 in 2023, and September 19 in 2024.
The determination of the dry matter content (%) in potato tubers was performed using the oven-drying method, while the starch content (%) was measured based on the determination of the specific gravity of the tubers using the Polikeit balance and the positive correlation between specific gravity, dry matter content and starch content.
The culinary quality characters (crushing on boiling, consistency, mealiness, and moisture content of the tubers, as well as the structure of starch granules) were evaluated through sensory analysis by the trained panellists, after the tubers were previously boiled at steam, at 100°C for 50 minutes. The tubers were uniform in size, shape and maturity.
The sensory analysis of culinary quality characters was conducted using a hedonic scale from 1 to 4 (Bărăscu et al., 2019, after Lugt and Goodijk, 1959), as follows:
- crushing on boiling: 1 = stay entire; 2 = crushing less; 3 = crushing; 4 = crushing more;
- consistency: 1 = very consistent; 2 = consistent; 3 = less consistent; 4 = uneven consistency;
- mealiness: 1 = waxy; 2 = less mealy; 3 = mealy; 4 = very mealy;
- moisture: 1 = wet; 2 = less humid; 3 = rather dry; 4 = dry;
- starch structure: 1 = fine granules; 2 = suitable fine granules; 3 = large granules; 4 = very large granules.
The scores obtained for each of the five characters were summed and were classified into value ranges corresponding to the following culinary use types: A (5.0-7.5); AB (7.6-10.0); B (10.1-12.5); BC (12.6-15.0). Potato tubers from types A and AB did not crumble, remained whole, were consistent, and had fine starch granules. These potatoes were ideal for salads but could also be used in various other dishes. Potato tubers from types B and BC were sufficiently firm, slightly crushed when boiled, had a mildly moist texture, and had a suitably fine structure of starch granules. Potatoes of this type were versatile and were suitable for various culinary applications.
The statistical analysis was conducted using Microsoft Excel and PAST 4 software, encompassing descriptive statistics, Pearson correlation analysis, and stability assessment based on environmental indices. Past 4 was used for the Pearson correlation coefficient matrix analysis and for generating mosaic plots, which display the obtained values using heat maps. The stability analysis of quality parameters was conducted using Microsoft Excel, following the method proposed by Eberhart and Russell (1966), which evaluates stability based on environmental conditions.

RESULTS AND DISCUSSIONS

Except for the year 2023, when a total rainfall of 419 mm was recorded during the critical period for potato cultivation, the crop faced drought periods in the other two years. These

drought conditions, combined with temperatures up to 3°C above the multiannual average, negatively affected not only plant growth and development but also the culinary quality and certain quality parameters of the tubers (Figure 1).

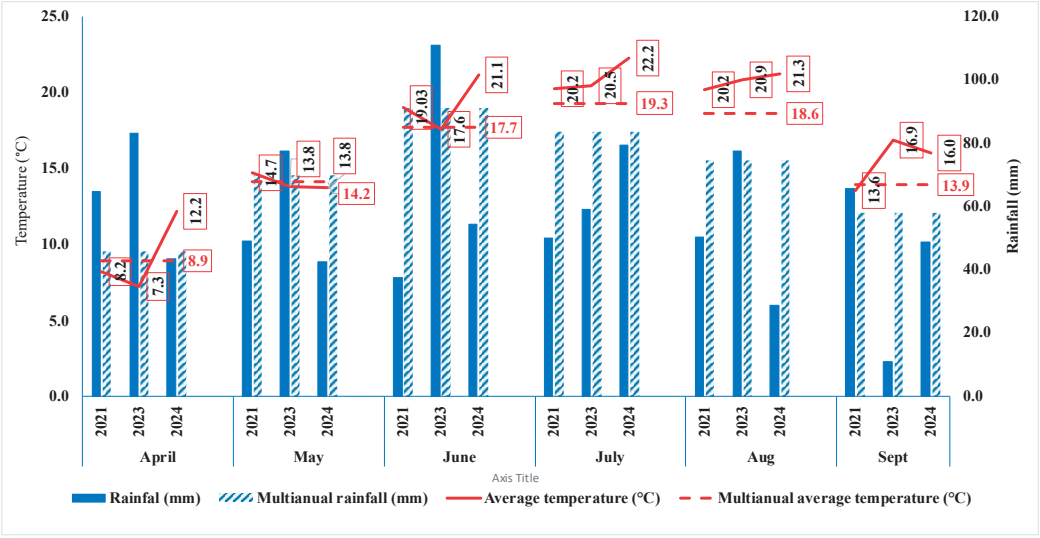


Figure 1. Temperatures and rainfall during the potato growing season (April-September, 2022-2024, Brașov)

During the three years of experimentation, the dry matter and starch content of the potato tubers were also determined. The dry matter content ranged from 16.8%, recorded in 2024 for the Ervant variety, to 24.1%, obtained for the Azaria variety in the first year of experimentation (Figure 2).

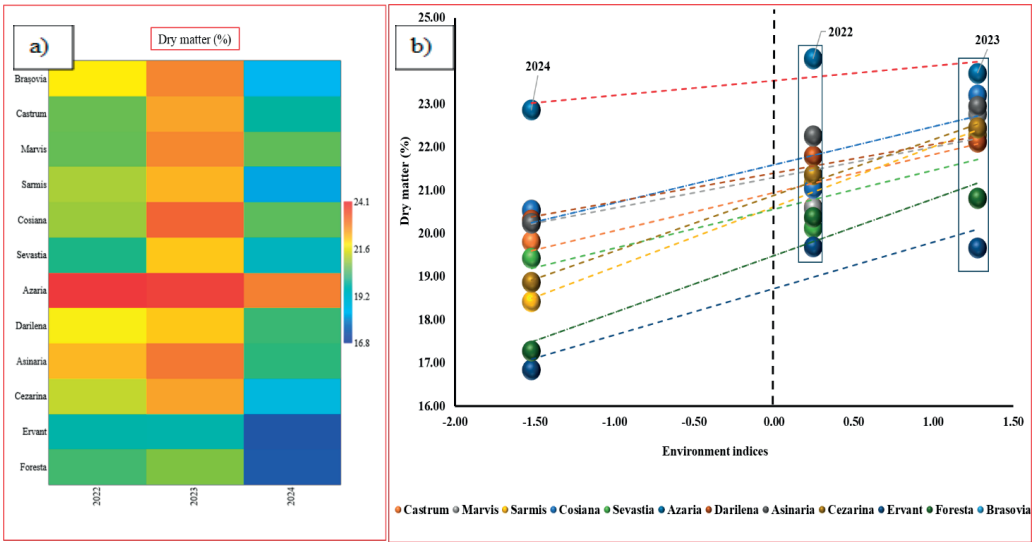


Figure 2. Dry matter content in potato tubers (a) and stability analysis of this quality parameter based on environmental indices (b)

The stability analysis of dry matter content, based on environmental indices obtained over the three years for the 12 potato varieties, suggests that higher values were recorded in

2022 and 2023, while 2024 proved unfavorable for dry matter accumulation. In terms of stability, Azaria variety stood out, showing pronounced stability, as indicated by the regression line being nearly parallel to the Ox axis (Figure 2).

Regarding starch content (Figure 3), values ranged from 10.1%, recorded for the Ervant, Foresta, and Sevastia varieties in 2024, as well as for Ervant in 2022, to 14.8%, achieved by Asinaria variety in 2023. The stability analysis of starch content, based on environmental conditions over the three years of experimentation, showed that, in general, higher starch accumulation occurred in 2023 and 2022, while 2024 was less favorable for this quality parameter in tubers. As Yong Zhen Ma et al., 2024 show in their research, soil water stress significantly reduced starch accumulation in potato tubers.

Regarding starch content stability over the three years, the Azaria and Ervant varieties stood out with lower fluctuations in values from year to year, indicating a more consistent performance across different environmental conditions.

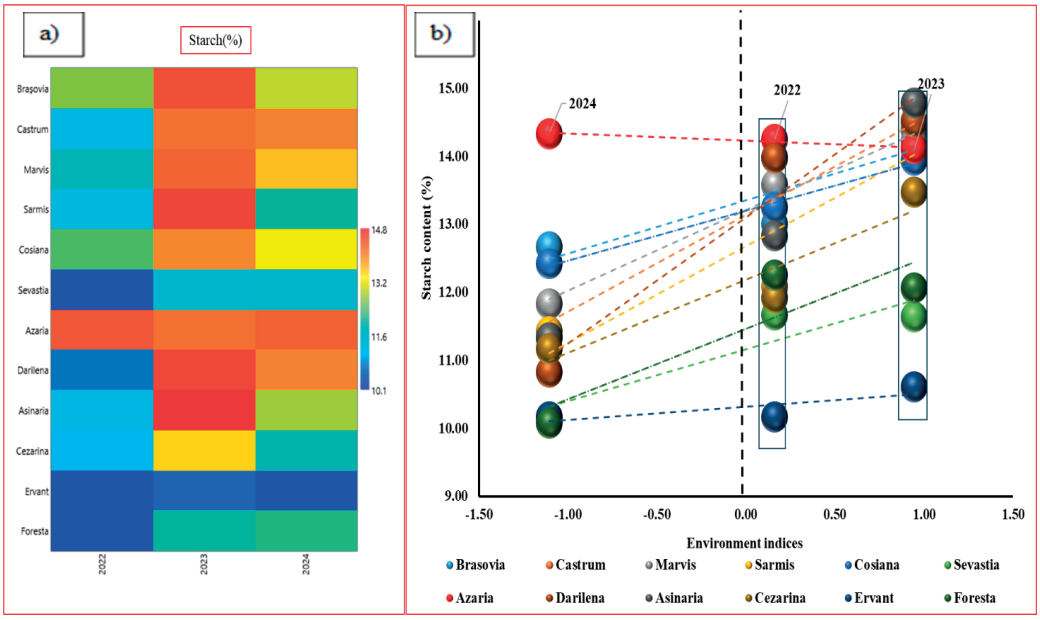


Figure 3. Starch content in potato tubers (a) and stability analysis of this quality parameter based on environmental indices (b)

The culinary quality of the studied biological material varied significantly, depended on both genotype and experimental year (Figure 4). Regarding crushing on boiling, scores ranged from 1 to 2.9, while consistency varied between 1 and 3.3. Mealy texture was evaluated between 1.1 and 3.1, moisture content had values ranging from 1.3 to 2.9, and starch structure varied between 1.3 and 2.8. Castrum variety received the highest score for crushing on boiling in 2023 (2.9) and for consistency in 2022 (3.3). In the second experimental year, mealy texture scores were highest for Cosiana, Azaria, and Cezarina varieties, with Azaria also achieving high scores for starch structure in 2023 and moisture content in the first two experimental years.

By summing the scores obtained for culinary quality indicators, the genotypes generally fall into the following usage types: A, AB, B and BC. Based on its performance over the three experimental years, Sevastia variety had the lowest total score for culinary quality indicators, which placed it in category A, regardless of the experimental year. This variety was characterized by firm potatoes, suitable for salads, with tubers that did not crumble, remained intact, were non-mealy, moist, and had a fine texture.

Sarmis, Cezarina, Ervant, and Foresta varieties, based on the average sum of their culinary quality scores, fell into the AB type, and Castrum and Azaria varieties fell into the BC type.

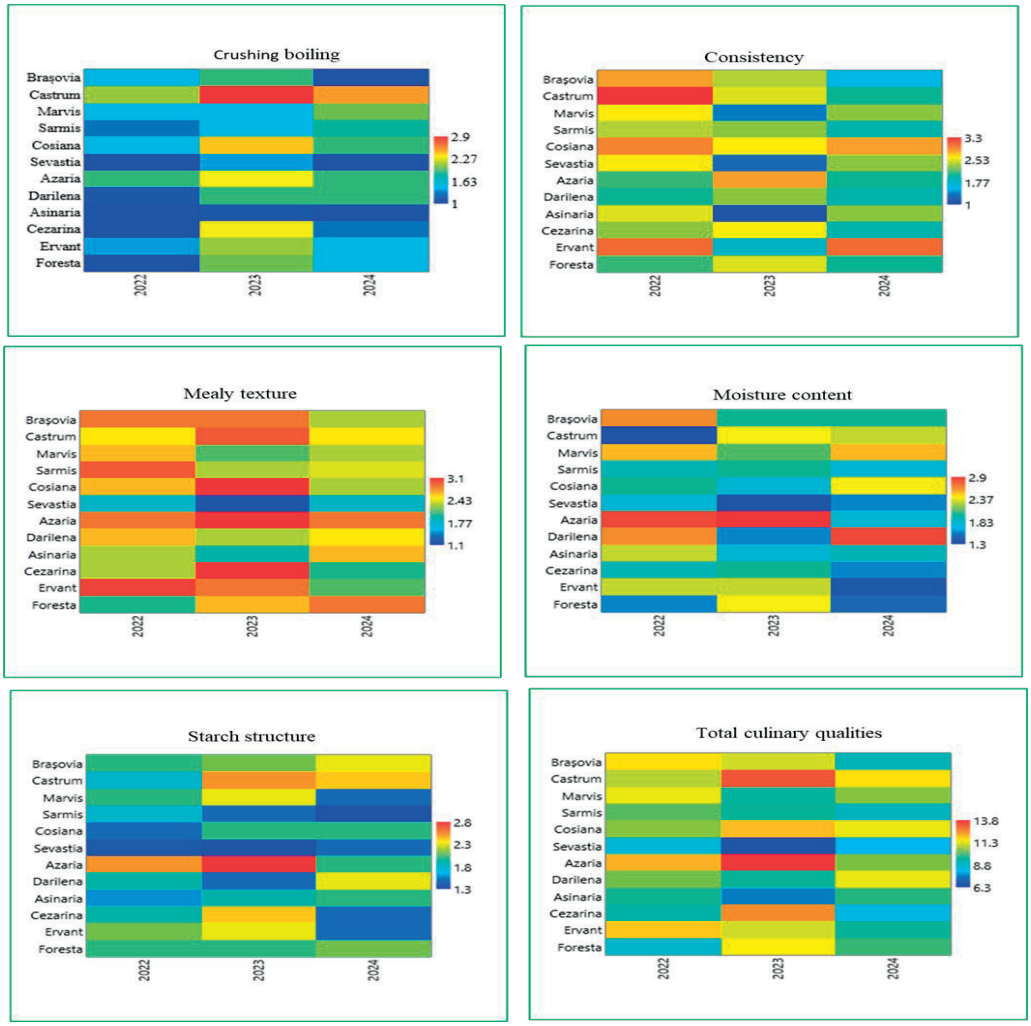


Figure 4. Culinary quality of the 12 potato varieties grown in three different years (2022, 2023, 2024) with distinct climatic conditions

Based on the calculated Pearson correlation coefficient matrix (Figure 5) for dry matter content, starch content, and culinary quality indices, a generally direct positive relationship between these parameters was observed.

Negative correlations were identified only between starch content and tuber consistency (r = -0.01) and between dry matter content and consistency (r = -0.06). High correlation coefficient values were obtained for the relationship between dry matter content and starch content (r = 0.81), as well as between crushing on boiling and mealiness (r = 0.53). Additionally, a strong positive correlation was observed between crushing on boiling and starch structure (r = 0.46), as well as between mealiness and moisture content (r = 0.48). Furthermore, a very strong direct positive relationship was established between mealiness and starch structure, with a calculated correlation coefficient of r = 0.64.

From the analysis of Figure 6, it was observed that the average culinary quality of the tubers from the 12 potato varieties was not significantly influenced by the total precipitation recorded during the growing season over the three years. Instead, it varied more depending on the variety and the interaction between varieties and environmental conditions, indicating that other factors, such as the genetic characteristics of the varieties and their adaptability to different environmental conditions, generally influenced the culinary quality of potatoes.

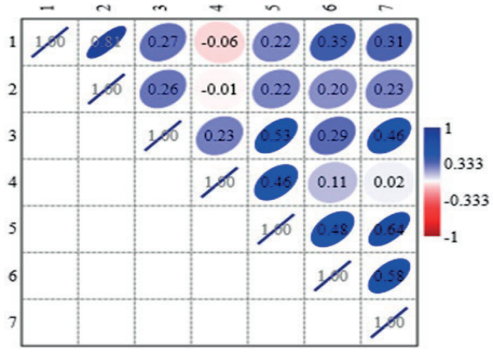


Figure 5. Correlation coefficient matrix for dry matter content (1), starch content (2), crushing on boiling (3), consistency (4), mealiness (5), moisture (6), starch structure (7)

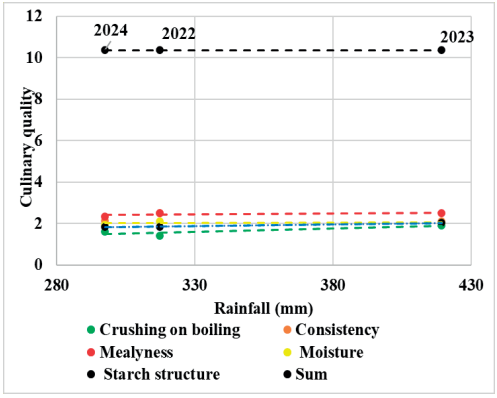


Figure 6. Culinary quality of potato tubers based on total rainfall

CONCLUSIONS

The climatic conditions during the growing season directly influence the technological parameters of potato tubers, dry matter and starch tubers content. Lower precipitation levels, leading to water stress, can decrease the starch content in potato tubers.

Azaria and Ervant varieties stood out with lower fluctuations in starch tuber content values from year to year, suggesting greater stability of this parameter under varying environmental conditions.

The average culinary quality of the tubers, crushing on boiling, consistency, mealiness, moisture content of potato tubers, and the structure of starch granules varied more depending on the variety and the interaction between varieties and environmental conditions. Careful management of environmental factors, together with the use of adapted potato varieties, can ensure the achievement of productions that meet the requirements for a specific type of use.

Future research should explore integrating genotypic data or irrigation management to further improve tuber quality stability under climate variability.

ACKNOWLEDGEMENTS

This research work was financed from Project PN 23 19 02 01/2023.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

MODERN MAIZE CULTIVATION TECHNOLOGIES IN THE CONTEXT OF CLIMATIC CHALLANGES

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Abstract

Maize is one of the most important agricultural crops globally duet o its higt productivity and various applications in food, feed and industry. The study was carried out in the area of gleic soils in the west of Romania and aimed to optimize the tillage system and test the performance of some maize hybrids in the context of climate change, comparing two tillage – minimum and conventional tillage systems – in terms of the impact on average production. The results obtained show that the minimum tillage system led to an increase in avarage production by 1,117 kg/ha compared to the conventional system. In the dry year 2022, the difference was 603 kg/ha, and in 2023, more climatically favorable, the difference was more pronounced, reaching 1,608 kg/ha. The technology of maize cultivation in a minimum tillage system and the adaptability of hybrids are the viable solution for increasing the harvest in unfavorable climatic conditions.

Key words*: corn hybrids, yield performance, sustainable agriculture.*

INTRODUCTION

Climate change is one of the greatest challenges for global agriculture, affecting maize production through temperature variations, water deficits, and increased frequency of extreme weather events (Lal, 2020). Maize (*Zea mays* L.), being one of the most widely cultivated crops worldwide, requires technological and genetic adaptations to cope with changing climatic conditions (Edmeades et al., 2017). Recent studies highlight that high temperatures and water deficits significantly reduce maize yields, affecting both quality and quantity of production (Cairns et al., 2013; Zhang et al., 2021). In semi-arid regions, the impact of climate change is particularly severe, necessitating the implementation of effective adaptation strategies (Tsubo et al., 2003). Erenstein et al. (2022) analyze global maize production trends and emphasize the need for investments in research and development to enhance drought resistance. Similarly, Schulz et al. (2020) suggest using intercropping techniques to optimize yields and conserve soil biodiversity. To combat the effects of climate change, researchers have developed multiple strategies, including genetic selection for drought tolerance, improved nitrogen management, and optimized planting dates (Chițu et al., 2024; Srivastava et al., 2018). The *Drought Tolerant Maize for Africa (DTMA)* program by CIMMYT (2020) demonstrates the success of genetic improvement in developing drought-tolerant maize hybrids. Additionally, Gheith et al. (2022) showed that efficient nitrogen application strategies enhance maize productivity and nitrogen use efficiency. FAO (2019) highlights the importance of climate-smart agriculture, promoting techniques such as mulching and efficient irrigation systems. Roman et al. (2011) analyze modern maize cultivation technologies and propose the use of hybrids adapted to changing climatic conditions. Intercropping, the simultaneous cultivation of maize with other plants, has proven beneficial for conserving soil moisture and improving soil fertility (Mousavi & Eskandari, 2011). For example, studies by Dawo et al. (2007) and Batugal et al. (1990) highlight the advantages of intercropping maize with legumes for yield enhancement and soil quality improvement. Nitrogen fertilization plays a crucial role in increasing maize crop productivity. Wei et al. (2019) demonstrated that plant density and the amount of nitrogen applied influence maize kernel filling parameters. Furthermore, Congreves et al. (2021) proposed redefining

nitrogen use efficiency to support sustainability in agriculture. Oraby et al. (2005) investigated the response of ten maize hybrids to different nitrogen fertilization levels, concluding that optimized nitrogen application improves yields under varying soil conditions. Additionally, Hu et al. (2020) demonstrated that split nitrogen application throughout the growing season increases maize yield in arid conditions.

Analysis of climate change effects across different geographical regions shows that impacts vary depending on local climatic conditions. For example, studies conducted in Romania by Smuleac et al. (2020) revealed difficulties in water accessibility for agricultural production in the Banat Plain. In Asia, Habib-ur-Rahman et al. (2022) identified key challenges and opportunities for the region's agriculture, emphasizing the necessity of effective adaptation policies. Climate change significantly impacts maize production, necessitating the implementation of effective adaptive strategies. Research suggests that genetic improvement, optimized nitrogen management, intercropping, and the adoption of sustainable agricultural practices can help maintain and improve agricultural yields under changing climate conditions (Lal, 2020; FAO, 2019). Therefore, it is essential for farmers, researchers, and policymakers to collaborate in implementing the most effective climate change adaptation solutions to ensure global food security (Erenstein et al., 2022).

MATERIALS AND METHODS

The research was carried out in the southwest of Timiș County, between the Bega Canal and the Timiș River, near the border with Serbia. The data regarding the monthly average temperatures during the experiment period are presented in Figure 1. From the analysis of the data, it can be seen that for the months of January, February and March the monthly average of temperatures in 2023 was higher than that of 2022, in April the monthly average in this period was very close, April was almost similar. Large temperature differences were recorded in May-July, where temperatures in 2022 were much higher compared to the same period in 2023. In the other months, there were no significant differences, except for October and December, which were warmer for 2022. Correlation coefficient: 0.96, which indicates a very strong relationship between the annual temperatures of 2022 and 2023. This means that the temperature patterns were similar between the two years.

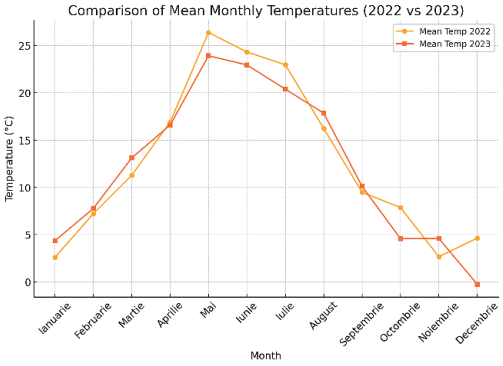


Figure 1. Comparison of the average monthly temperatures during the experimentation period

The correlation between the air temperature and the BBCH stage requirement for maize is shown in Figure 2.

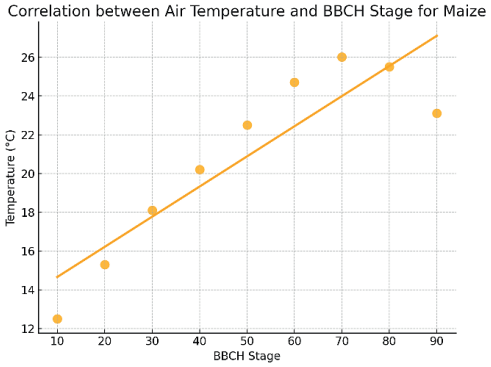


Figure 2. Correlation between monthly average temperatures and BBCH stage requirement for maize

The correlation coefficient is 0.90, which shows a strong correlation between air temperature and the BBCH stage, indicating an increase in temperature as the corn advances in development.

The average monthly rainfall recorded during the experimental period is shown in Figure 3.

In January, April and September, higher amounts of precipitation were recorded for 2022 compared to 2023. In March, June and July we had a rainfall deficit for 2022, and in 2023 the

monthly average rainfall was enough for the plants not to suffer water stress. A very large difference in the average rainfall is observed, in 2022 the average being 108.8 mm lower than in 2023. The correlation between the monthly rainfall during the experimental period and the BBCH stage of maize development is shown in Figure 4.



Figure 3. Average monthly rainfall recorded during the experimental period

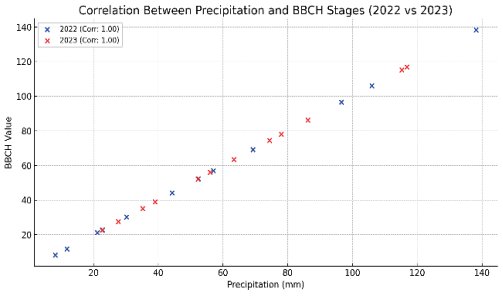


Figure 4. Correlation between the monthly rainfall during the experimental period and the BBCH stage of maize development

BBCH stage 00-19 - March - April (emergence and beginning of vegetative development). In 2022 there was higher rainfall in April, which favoured a good start of the crop, and in 2023 rainfall was lower in April, which led to a later emergence and a more difficult start. BBCH stage 20-39 -May - June (vegetative growth), due to less rainfall in this interval of 2022, water stress was recorded, while in 2023 the better distributed rainfall in May and June favoured plant growth.

BBCH stage 50-69 -July - August (flowering, pollination and berry formation), very low rainfall in July 2022 influenced pollination and grain formation and implicitly the grain production yield. In 2023, there was more rainfall in July, which favoured a better grain yield. In conclusion, 2023 benefited from a more favourable distribution of rainfall, which explains a higher BBCH in certain months compared to 2022, therefore a better growth and development with direct implications on the level of production.

The type of soil on which the study was carried out was gleiosol with a clay-clay to clayey texture, with a grainy to polyhedral structure, high humus content with a moderate - low acidic pH. Among the negative qualities to be noted is the degree of compaction. Under these conditions, soil loosening works are mandatory, in order to improve the aero-water regime. The experiments were bifactorial, in which *factor A* represented the tillage, with two graduations and *factor B* the hybrid cultivated with 7 graduations. Gradations *of factor A - tillage:* a1 - tillage in the "minimum tillage" system; a2 - tillage in the "conventional system". The tillage of the soil in the minimum tillage system was: after harvesting the wheat, when the soil moisture allowed, the scarification work was done directly at a depth of 50-70 cm and with a distance between the arms of the scorer of 70 cm. The work was carried out on both diagonals, thus ensuring the loosening of the soil and the destruction of the hardpan. After ensuring good humidity, a work was carried out with the tiger, at a depth of 20 cm, through this work the stubble was incorporated into the soil.

In the variant (2) in which the conventional system was applied, the works carried out were as follows: the first disc harrow work for the destruction of the stubble and the conservation of water in the soil, the second work was the plough work at a depth of 25 cm., the third work - disc cultivator + rotary harrow for the preparation of the seedbed.

Factor B represented the maize hybrid, using 7 hybrids, with different vegetation periods, as follows: the Torino hybrid of the FAO 320 group; the DKC4391 hybrid of the FAO 340 group; the P9537 hybrid of the FAO 350 group; the DKC 5092 hybrid; the DKC 5182 hybrid of the FAO 400 influenza; the P0170 hybrid of the FAO 450 group and the Fabio hybrid of the FAO 460 group.

The hybrids taken in the study are new products produced by Bayer, Syngenta and Corteva companies. Fertilization was carried out with 126 kg/ha N, 65 kg/ha P_2O_5 and 84 kg/ha K_2O. The sowing density was 72,000 germinating

seeds/ha, the sowing was done at a distance of 70 cm between the rows and 5 - 6 cm deep.

RESULTS AND DISCUSSIONS

The results presented in the paper were calculated according to the calculation method for bifactorial experiments, with 3 repetitions. For a better example of the influence of the soil tillage system in conditions of water stress and atmospheric heat, we present the results obtained per experimental year.

Table 1. Shows the harvest results obtained in 2022

Factor B Hybrid	Factor A - systems of works		Average Yield kg/ha
	A1-minimum tillage kg/ha	A2-conventional kg/ha	
Torino	4033	3523	3778
DKC4391	6662	5819	6240
P9537	4945	3730	4337
DKC5092	6610	5830	6220
DKC5182	4462	4800	4631
P0170	5332	5025	5178
Fabio	6977	6071	6524
X	5574	4971	5272

Average Factor A

Systems of works	Yield kg/ha	%	Difference kg/ha	Significance
A1	5574	100		
A2	4971	89	-603	000
DL5%=157 DL1%=261 DL0.1%=488				

Average Factor B

Hybrid	Yield kg/ha	%	Diff. kg/ha	Significance
Torino	3778	100		
DKC4391	6240	165	2462	XXX
P9537	4337	115	559	XXX
DKC5092	6220	165	2442	XXX
DKC5182	4631	122	853	XXX
P0170	5178	137	1400	XXX
Fabio	6524	172	2746	XXX
DL5%=151 DL1%= 212 DL0.1%=300				

From the data presented in Table 1, it can be seen that the harvests obtained for all the experimented hybrids were lower than the potential of these hybrids, ranging from 4033 kg/ha at Turin to 6972 kg/ha at Fabio in the tillage minimum tillage system and between 3523 kg/ha and 6071 kg/ha at the same hybrids cultivated in the conventional system. On average, on the 7 hybrids researched, the harvest was 5574 kg/ha in the minimum tillage system and 4971 kg/ha in the conventional one.

It follows that the harvest in the conventional system represented only 89% compared to the minimum tillage, returning a difference of -603 kg/ha, statistically ensured as very significantly negative.

From the analysis of the behaviour of the hybrids, on average on the two agrofunds, the lowest harvest of 3778 kg/ha was recorded in the Turin hybrid (FAO 320), the earliest of the cultivated ones.

In the hybrids of the groups between FAO 340 and 370, average harvests of over 6000 kg/ha were recorded, in the hybrid DKC 4391 and the hybrid DKC 5092, and in the hybrid P 9537, the harvest was only 4337 kg/ha. The semi-late hybrids of the 400 - 500 group achieved harvests of 4631 kg/ha at the DKC 5182 hybrid, 5178 at P0170 and 6524 at Fabio.

The average harvests on the two systems compared to the Turin harvest are higher with very significant differences.

The results obtained this year highlight the importance of the soil tillage system that allows water to be stored and reduces its loss from the soil through repeated mechanical works. Also, the good behaviour of 3 hybrids that exceeded 6000 kg/ha and can be considered tolerant to the harsh climatic conditions of this year is highlighted, in which in the critical months of the vegetation period only 11.8 mm of precipitation were recorded in June and 8.2 mm of precipitation in July and maximum temperatures of over 300C were also recorded in the mentioned period.

In conclusion, the results of the dry year in which the development of important vegetation phases were carried out in conditions of water and heat stress, due to the application of technologies favourable to the crop, still obtained acceptable results.

This year was favourable for the corn crop, the rainfall during the vegetation period of 86.2 mm in April, 115.2 mm in May, 52.2 mm in June, 27.6 mm in July and 78 mm in August explains that the harvests were on average on cultivated hybrids of 9783 kg/ha in plots worked in the minimum tillage system and 8175 kg/ha in the conventional system. The harvest difference of -1608 kg/ha in favour of the minimum tillage system is very significantly negative.

Table 2. Shows the harvest results for 2023

Factor B Hybrid	Factor A - systems of works		Average yield kg/ha
	A1- minimum tillage kg/ha	A2- convențional kg/ha	
Torino	9209	7733	8471
DKC4391	10095	8025	9060
P9537	9624	7824	8724
DKC5092	9419	8116	8767
DKC5182	9917	8117	9017
P0170	10185	8702	9443
Fabio	10036	8708	9372
X	9783	8175	8979

Average Factor A

Systems of works	Yield kg/ha	%	Difference kg/ha	Significance
A1	9783	100		
A2	8175	83	-1608	000
DL5%=76 DL1%=126 DL0.1%=237				

Average Factor B

Hybrid	Yield kg/ha	%	Diff. kg/ha	Significance
Torino	8471	100		
DKC4391	9060	106	589	XXX
P9537	8724	102	253	XX
DKC5092	8767	103	296	XXX
DKC5182	9017	106	546	XXX
P0170	9443	111	972	XXX
Fabio	9372	110	901	XXX
DL5%=146 DL1%= 206 DL0.1%=290				

The highest harvests of over 10,000 kg/ha were recorded in hybrids DKC4391 (10,095 kg/ha), P0170 (10,185 kg/ha) and Fabio hybrid (10,036 kg/ha) grown in the minimum tillage system (Table 2). It should be noted that in the conventional system there were no harvests higher than 8,708 kg/ha.

This year's results show that corn is a crop that capitalizes, in normal years, on the productive potential of the area. Better productivity is obtained if in the minimum system of works the basic work is presented by scarification at a depth of 70 cm, the favourable effect of this work being maintained in years 2 and 3 compared to the date of execution.

The average harvest on the two systems shows that the best results are obtained by cultivating the hybrids DKC 4391 (9060 kg/ha), DKC5182 (9017 kg/ha), P0170 (9443 kg/ha) and Fabio (9372 kg/ha).

Crop differences are statistically assured as very significant, except for the P9537 hybrid, where the difference is distinctly significant. In conclusion, in the climatically favourable years of all 7 cultivated hybrids, harvests of over 9,000 kg/ha were obtained in the minimum tillage cultivation system, which shows their good productive biological potential.

Table 3 summarizes the yield results obtained in 2022-2023.

The synthesis of the results presented allows the conclusion to be drawn regarding the behavior of the investigated hybrids in different climatic conditions.

Under the given conditions, the differences between the two systems of works are obvious. Thus, in the minimum tillage system, the hybrids managed to achieve economically efficient harvests between 6,600 kg/ha and 8,507 kg/ha.

Table 3. Summary of harvest results from the 2022-2023 experimental cycle

Factor B Hybrid	Factor A - systems of works		Average yield kg/ha
	A1- minimum tillage kg/ha	A2- convențional kg/ha	
Torino	6621	5628	6124
DKC4391	8378	6922	7650
P9537	7284	5777	6530
DKC5092	8011	6972	7491
DKC5182	7189	6458	6823
P0170	7758	6863	7310
Fabio	8507	7389	7948
X	7678	6572	7125

Average Factor A

Systems of works	Yield kg/ha	%	Difference kg/ha	Significance
A1	7678	100		
A2	6572	85	-1117	000
DL5%= 114 DL1%=189 DL0.1%=354				

Average Factor B

Hybrid	Yield kg/ha	%	Diff. kg/ha	Significance
Torino	6124	100		
DKC4391	7650	124	1526	XXX
P9537	6530	106	406	XXX
DKC5092	7491	122	1367	XXX
DKC5182	6823	111	699	XXX
P0170	7310	119	1186	XXX
Fabio	7948	129	1824	XXX
DL5%=107 DL1%=150 DL0.1%=212				

Under conventional system cultivation, the separation of harvests for the investigated hybrids was between the limits of 5,628 kg/ha and 7,389 kg/ha. The average difference

between the harvest of hybrids grown in the minimum tillage system and the conventional one is -1117 kg/ha, a very significantly negative difference, with harvests of over 8,000 kg/ha in the minimum tillage working system were the hybrid Fabio with 8,507 kg/ha, DKC 4391 with 8,378 kg/ha and DKC 5092 with 8,011 kg/ha. It should be noted that on average over the two years no hybrid was below 6,700 kg/ha. In the conventional working system, the Fabio hybrid with the highest harvest was 7,389 kg/ha, and the Turin hybrid with the lowest harvest, with 5,628 kg/ha. It should be noted that 4 of the 7 hybrids were with harvests between 6,000 and 7,000 kg/ha.

The analysis of the average harvests on the two working systems shows that 4 hybrids recorded harvests between 7,300 and 7,900 kg/ha. The entire assortment of hybrids researched motivates the continuation of research in the coming years, because the average level of harvests exceeds 6,000 kg/ha, proving that they have a high biological potential.

CONCLUSIONS

The research carried out in the area of gleic soils in the west of the country, on corn cultivation, aimed to make a contribution to the adaptation of the tillage system and the testing of new corn hybrids, in the conditions of climate change that negatively affects harvests. The researches regarding the tillage carried out in two variants - the tillage in the "minimum tillage" system and the tillage in the conservative system, on average over the two experimental years, showed the superiority of the minimum tillage system in which the average harvest, on the 7 hybrids taken in the study, was higher by 1,117 kg/ha. The harvest differences between the two working systems were only 603 kg/ha in the dry year 2022 and 1,608 kg/ha in 2023, more climatically favourable due to the rainfall that fell both in terms of quantity and monthly distribution, but without being enough for the corn needs. With reference to the behaviour of the 7 hybrids studied (Torino, DKC4391, P9537, DKC 5092, DKC 5182, P0170, Fabio) the average harvest in the two experimental years was 7678 kg/ha in the minimum tillage system and 6572 kg/ha in the conventional tillage system.

The work presented is highly topical due to the serious climatic deviations felt more and more in recent decades with effects on harvests and respectively on ensuring people's nutrition.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

INFLUENCE OF TILLAGE AND VARIETY ON YIELD AND QUALITY OF CHICKPEA CROP ON THE REDDISH PRELUVOSOL FROM MOARA DOMNEASCĂ, ROMANIA

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Abstract

In grain legume crops, cultivated varieties and basic tillage are the first important factor for improving yield and quality indicators, especially in recent years when climate change has induced long dry periods during the growing season. Thus, the research focused on the influence of tillage and chickpea varieties on production, productivity elements and quality indicators in the reddish preluvosol zone, knowing the less favorable characteristics of the soil type. The research was carried out over two agricultural years period respectively, 2022-2024 under climatic environment of Moara Domneasca, Ilfov county. Three tillage variants were evaluated: plowing at 25 cm depth, chiseling at 35 cm depth and disc harrowing at 12 cm depth and 4 chickpea varieties: Burnas, Rodin, Flamenco and CDC Orion. The results obtained showed differences between the varieties of chickpeas taken in the study but especially between the tillage. The highest yield was obtained by CDC Orion variety in the experimental trial in which the soil was ploughed, but Flamenco variety had a higher protein and lipid content in the kernels.

Key words*: chickpea varieties, tillage, yield, quality.*

INTRODUCTION

The chickpea is the grain legume crop with an increasing global demand in recent years (Grasso et al., 2022). It is recommended in sustainable agriculture because it requires less water amount and improves soil characteristics (Millán et al., 2015).

Chickpea is suitable in crop rotation causing an increase in soil fertility, being a very good previous crop for winter cereals by biological nitrogen fixation from the *Rhizobium leguminosarum* subsp. *ciceri* bacteria symbiosis (Moldovan and David, 2010; Singh and Singh, 2018; Gebremariam and Tesfay, 2021). This limits the negative effect on environment and soil by reducing the quantities of chemical fertilizers per hectare.

Another very important quality of chickpeas is due to its protein quality, being one of the best in the grain legume family (Sotelo et al., 1987). It has an amino acid composition characterized by a high content of leucine, lysine, valine, isoleucine and with amino acids in lower amount as cysteine and methionine (Gutium and Ciumac, 2012).

Chickpea kernels are rich in many nutrients such as: good quality protein around 23.81%, fat around 6.57%, sugars around 3.94%, starch around 50.36%, cellulose around 9.01% (Gutium and Ciumac, 2012) but also contain antinutritional elements around 3.10% (Sotelo et al., 1987). Besides these, the kernels contain iron, potassium, magnesium, calcium, phosphorus, zinc, vitamin B complex, vitamin C and vitamin K and are high in fiber (Jukanti et al., 2012). More than nutritional importance, chickpea kernels have remarkable medicinal properties, enrich blood and cure skin diseases (Warrier et al., 1995). They also have antihelmintic properties and settle the functioning of the digestive tract (Sastry and Kavathekar, 1990; Pittaway et al., 2006; Zia-Ul-Haq et al., 2008).

In grain legume crops, cultivars and tillage are the main important factors for improving yields and quality indicators, especially in recent years due to climatic changes inducing long dry periods during the growing season.

Tillage practices change soil physical properties as soil structure, mechanical impedance, dry bulk density, thermal conductivity, pore size distribution, water distribution, root distribution and production output (Comia et al., 1994; Abu-Hamdeh, 2000; Tapela & Colvin, 2002; Barzegar et al., 2003). Each tillage operation is effective in the soil in different ways. Changes in soil physical properties that increase the pore space lead to improved crop performance but with no tillage they are reduced (Barzegar et al., 2003).

Adequate strategies are essential to optimize the potential and benefits of chickpea varieties from diverse agro-ecological environments and incorporated them into other cropping systems. Lack of adequate information, often leads to their placement in the wrong target regions, where they have had partial success (Shiyani et al., 2002).

The research focused on the comparison of different chickpea varieties and different tillage systems on yield, productivity and quality indicators under pedoclimatic conditions at the Moara Domnească Agronomic Didactic Station, Ilfov County, in the reddish preluvosoil zone. Four chickpea varieties were assessed: Burnas, Rodin, Flameco and Orion and three tillage variants: plowing at 25 cm depth, chisel at 35 cm and disk harrow at 12 cm.

MATERIALS AND METHODS

Research was carried out during 2023 and 2024, at the Moara Domnească Agronomic Didactic Station, Ilfov County. The experimental field was located on different plots depending on the year, the geographical coordinates where the experiment was located. For 2023 it was 44,50294 latitude N and 26,24996 longitude E, and for 2024 was 44.500665 latitude N and 26,249435 longitude E, on the soil type reddish preluvosol belonging to the luvisol class, which is dominant in this region. The research on this soil type are important because the less favorable characteristics of the soil type are known, in this case with a high clay content above 32% and low humus content near 2.2%, a moderately acidic pH around 5.4 and with a low water permeability, with crust forming immediately after precipitation (Mihalache et al., 2009; Mihalache et al., 2010; Mihalache et al., 2014).

The experiment was conducted in a randomized block design with 3 replications, with 12 variants with an experimental area of 21 m^2. The experimental variants consisted of plow at 25 cm depth, chisel at 35 cm depth and disk harrow at 12 cm depth, on which Burnas, Rodin, Flameco and Orion varieties were sown.

The chickpea varieties used in the study have different origins, so Burnas and Rodin are of Romanian origin. They were obtained at Agricultural Research and Development Station Teleorman and approved in 2006, being suitable for growing in arid areas of our country. Research carried out in south-western Romania showed a high raw protein content in Burnas variety around 24.9% and in Rodin around 23.8% (David et al., 2010). The chickpea variety Flamenco has French origin registered in 1991. It is a highly plant dropping and grain shattering resistant, anthracnose resistant, drought and cold tolerant variety with high yield potential and high protein content. The Orion variety is of Canadian origin registered in 2010 and developed by the Crop Development Centre, University of Saskatchewan (Taran et al., 2011). It is a large grain size variety being visually appealing for market marketing. It is highly drought resistant, anthracnose tolerant and has a very high yield potential.

Tillage was done in late November and sowing of the chickpea varieties was done on April 3 in 2023 and March 29 in 2024. Before sowing, the soil was tilled with the cultivator. The chickpea varieties taken in the study were sown after the winter wheat crop, at a density of about 40 plants/m^2, row spacing of 50 cm and an average depth of 5 cm.

The determination of proteins (%) and lipids (%) was carried out in the Phytotechnics Laboratory of the Faculty of Agriculture, USAMV of Bucharest, using an infrared spectrophotometer, Instalab NIR Product-Analizer. The analyzed samples were uniformly ground using a laboratory mill and determined on the same day. Measurements for the quality indicators as 1000 seeds weight (TSWG) and hectoliter weight (HW) were carried out in the Agrotehnics laboratory of the Faculty of Agriculture, USAMV of Bucharest.

Weight per 1000 kernels (TSWG) is a quality indicator for the chickpea crop because large-sized chickpeas are often required.
Productivity indicators such as number of main branches, number of pods/plant, number of kernels/plant, number of kernels/pod, and weight of kernels/plant (g) were determined.
Statistical data were interpreted by analysis of variance, and LSD 5% was used to perform multiple comparisons (Săndoiu, 2020).

RESULTS AND DISCUSSIONS

In terms of precipitation during growing season (March to August), in 2023, chickpeas crop benefited 242 mm, that was a dry year compared to the multi-year average recorded in Romania. In the first month of the growing season precipitation level was very low at only 14 mm but the following April was better with 72.5 mm. The months of May and June did not bring satisfactory rainfall while temperatures were high, these were 32.5 and 32 mm. A total of 91 mm was accumulated for the months of July and August.

Rainfall during the growing season of the 2024 was identical to the previous year in terms of total precipitation, totalling 241.1 mm, but it was distributed differently. In the first part, the rainfall amount was favorable with 59.5 mm in march and 43 mm in April, but after that the drought occurred, with 22.2 mm in May and 15 mm in June. The hot summer months of July and August amounted to 101.4 mm (Figure 1).

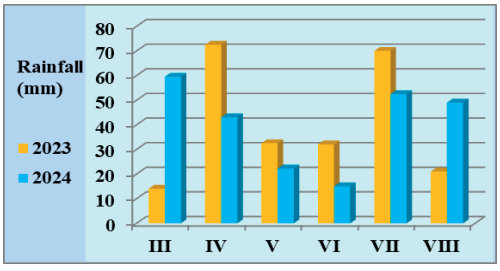


Figure 1. The evolution of rainfall at Moara Domnească, 2023-2024

Regarding temperatures, the two years were roughly similar, with very high temperatures and serious drought. The highest recorded temperatures were in August for the year 2023 and in July for the year 2024 with average monthly temperatures of 26.8 and 26.9°C respectively. The year 2024 had higher temperatures in June of 25.9°C compared to 21.7°C for the year 2023 (Figure 2).

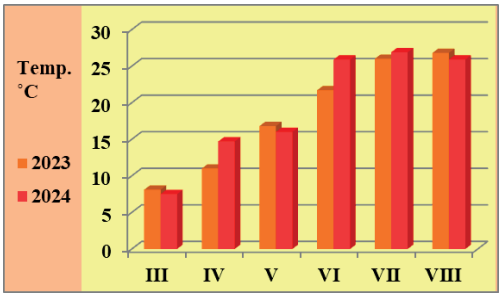


Figure 2. The evolution of temperature at Moara Domnească, 2023-2024

Due to unevenly distributed rainfall shortages in 2023 and 2024, as well as extremely high growing season temperatures above the annual average recorded at the station, the chickpea crop was affected by the water deficit. The chickpea crop is tolerant to arid weather, but for high yields, rainfall needs to be favorably distributed.
The results on the influence of cultivated variety and tillage on yield of chickpea crop of the research years 2023-2024 are shown in Table 1. From the data analysis, it can be noted that yields varied greatly in terms of tillage and less in terms of cultivated varieties. The crop years 2023-2024 were dry and the yields obtained were not high. The average yield of the cultivated varieties for the variant where the plowing was used, was 1566.0 kg/ha. Comparing with the chisel variety, where the average yield of the varieties was 1319.3 kg/ha and the disk variety 759.5 kg/ha, we can say that the yield of the basic plowed tillage was satisfactory in such conditions of drought.
The yield differences between plow, chisel and disking were -246.7 kg/ha and -806.5 kg/ha, being highly significantly negative. The 25 cm deep plowing plot benefited more from soil moisture at sowing time.
Comparing the yields of the studied varieties, the Orion variety had an average yield of 1272.8 kg/ha and in the plowed version being 1654.0 kg/ha, very significantly higher than the Burnas variety. The Flamenco variety had an average yield of 1265.8 kg/ha and with a very significant yield in the plowed variety of 1636.0 kg/ha. The

Burnas and Rodin varieties gave slightly lower average yields of 1163.8 kg/ha and 1157.2 kg/ha and with 1501.5 kg/ha and 1472.5 kg/ha respectively, in the plowed version.

In all tillaged variants, Orion and Flamenco performed the highest yields, with average yield difference of 109.0 and 102.0 kg/ha respectively, more than the reference variety Burnas.

Table 1. Influence of cultivated variety and tillage on chickpea yield, average of research years 2023-2024

No. crt.	Tillage	Variety	Yield		Dif. kg/ha	Significance
			kg/ha	%		
1	PLOWING	Burnas	1501.5	100.0	-	-
2		Rodin	1472.5	98.1	-29.0	-
3		Flamenco	1636.0	109.0	134.5	***
4		Orion	1654.0	110.2	152.5	***
Plowing average			**1566.0**	**100.0**	-	-
5	CHISEL	Burnas	1267.5	100.0	-	-
6		Rodin	1276.0	100.7	8.5	-
7		Flamenco	1357.5	107.1	90.0	***
8		Orion	1376.0	108.6	108.5	***
Chisel average			**1319,3**	**84.2**	**-246,7**	ooo
9	DISC HARROW	Burnas	722.5	100.0	-	-
10		Rodin	723.0	100.1	0.5	-
11		Flamenco	804.0	111.3	81.5	***
12		Orion	788.5	109.1	66	**
Disc harrow average			**759,5**	**48.5**	**-806.5**	ooo
Varieties average		**Burnas**	**1163.8**	**100.0**	-	-
		Rodin	**1157.2**	**99.4**	**-6.6**	-
		Flamenco	**1265.8**	**108.8**	**102.0**	***
		Orion	**1272.8**	**109.4**	**109.0**	***

Factor A: LSD 5% = 66.98 kg/ha; LSD 1% = 113.34 kg/ha; LSD 0.1% = 195.78 kg/ha.
Factor B: LSD 5% = 24.67 kg/ha; LSD 1% = 33,61 kg/ha; LSD 0,1% = 49.76kg/ha.
Factor A1/B2: LSD 5% = 42.27 kg/ha; LSD 1% = 57.76 kg/ha; LSD 0.1% = 77.41 kg/ha.

Statistical analysis of the results showed that the Orion and Flamenco varieties of the experimental study in which the main tillage was plowing, recorded the highest yields being found distinctly significant with values located in the difference class "a" with 1654.0 and 1636.0 kg/ha, respectively (Table 2).

Table 2. Analysis of the influence of tillage and cultivated variety on yield of chickpea crop, multiple comparisons, average of research years 2023-2024

Position no.	Variant	Yield (kg/ha)	Value group
I	Plowing/Orion (a1b4)	1654.0	a
II	Plowing/Flamenco (a1b3)	1636.0	a
III	Plowing/Burnas (a1b1)	1501.5	b
IV	Plowing/Rodin (a1b2)	1472.5	b
V	Chisel/Orion (a2b4)	1376.0	c
VI	Chisel/Flamenco (a2b3)	1357.5	c
VII	Chisel/Rodin (a2b2)	1276.0	d
VIII	Chisel/Burnas (a2b1)	1267.5	d
IX	Disc harrow/Flamenco (a3b3)	804.0	e
X	Disc harrow/Orion (a3b4)	788.5	e
XI	Disc harrow/Rodin (a3b2)	723.0	f
XII	Disc harrow/Burnas (a3b1)	722.5	f

Factor A1/B2: LSD 5% = 42.27 kg/ha; LSD 1% = 57.76 kg/ha; LSD 0.1% = 77.41 kg/ha.

The influence of cultivated variety and tillage on productivity parameters in chickpea crop for the average of the research years 2023-2024, such as number of main shoots/plant, total number of

pods/plant of which fertile and sterile, number of kernels/plant, number of kernels/pod and weight of kernels/plant are presented in Table 3. The mean number of main shoots/plant had the highest values in the plow tillage being on average 4.00, followed by chisel with mean value of 3.65 and the least developed plants were in the disc harrowed plot variety with mean values of 2.70. In terms of varieties influence, Orion exceeded the other varieties with mean values of 3.93, followed by Flamenco with 3.57, Rodin with 3.15 and Burnas with 3.07.

The average number of pods/plant was influenced more by the tillage than by the variety sown. The average number of pods/plant was 17.95 pods/plant in the plowing tillage, of which 2.35 were sterile pods, with a percentage of 13.09%, similar to the chisel tillage but lower than the disc harrowing tillage. In chisel plot, the number of pods per plant decreased to 16.95 and the number of sterile pods increased to 2.25 with a percentage of 13.27%. Thus, the lowest number of pods/plant was recorded in the disc harrow, on average 10.60 of which 2.60 sterile pods with a 24.53%. In terms of varieties, Orion had the lowest number of pods/plant of 13.82 but also the lowest number of sterile pods of 2.00. It was compared to Flamenco, which had 14.55 pods/plant of which 2.27 sterile pods, followed by Rodin with 15.99 pods per plant of which 2.58 sterile pods and then Burnas with 16.22 pods/plant of which 2.68 sterile pods.

The average number of kernels/plant was influenced more by the tillage than by the chickpea variety. In plow tillage, it was 19.80 kernels/plant, in chisel tillage it was 18.20 kernels/plant and in disc harrow tillage it was very low at 10.55 kernels/plant. The influence of the variety on the number of kernels/plant highlights the varieties Rodin and Burnas with 17.35 and 17.13, followed then by Flamenco with 15.42 and least in Orion with 14.78. Due to the characteristics of Orion and Flamenco varieties to have large kernel size, it resulted in better yield compared to Burnas and Rodin varieties which have much smaller kernel size.

Table 3. Influence of cultivated variety and tillage on chickpea productivity parameters, average of research years 2023-2024

No. crt.	Tillage	Variety	No. main shoots	No. pods/plant			No. kernels/ plant	No. kernels/ pod	Weight kernels/ plant (g)
				Total	Fertile	Sterile			
1	PLOWING	Burnas	3.65	19.35	16.35	3.00	21.35	1.11	5.05
2		Rodin	3.65	18.95	16.30	2.65	21.30	1.14	4.95
3		Flamenco	4.05	17.40	15.55	1.85	18.85	1.09	5.45
4		Orion	4.60	15.95	14.10	1.85	17.65	1.11	5.55
Plowing average			**4.00**	**17.95**	**15.55**	**2.35**	**19.80**	**1.11**	**5.25**
5	CHISEL	Burnas	3.25	18.15	15.55	2.60	19.10	1.05	4.55
6		Rodin	3.35	18.15	15.55	2.60	19.70	1.09	4.60
7		Flamenco	3.75	16.05	14.05	2.00	17,25	1.08	4.85
8		Orion	4.05	15.30	13.55	1.75	16.80	1.10	4.75
Chisel average			**3.65**	**16.95**	**14.65**	**2.25**	**18.20**	**1.08**	**4.70**
9	DISC HARROW	Burnas	2.30	11.15	8.70	2.45	10.95	0.98	2.55
10		Rodin	2.45	10.85	8.35	2.50	11.05	1.02	2.60
11		Flamenco	2.90	10.15	7.20	2.95	10.15	1.00	2.85
12		Orion	3.15	10.20	7.80	2.40	9.90	0.97	2.95
Disc harrow average			**2.70**	**10.60**	**8.00**	**2.60**	**10.55**	**0.99**	**2.75**
Varieties average		**Burnas**	**3.07**	**16.22**	**13.53**	**2.68**	**17.13**	**1.05**	**4.05**
		Rodin	**3.15**	**15.99**	**13.40**	**2.58**	**17.35**	**1.08**	**4.05**
		Flamenco	**3.57**	**14.55**	**12.27**	**2.27**	**15.42**	**1.06**	**4.38**
		Orion	**3.93**	**13.82**	**11.82**	**2.00**	**14.78**	**1.06**	**4.42**

The average number of kernels in the pod for plowing and chiseling was 1.11 and 1.08 respectively and for disk harrowing 0.99. The influence of varieties on the average number of kernels in the pod is irrelevant, with very small differences. The kernels weight per plant was influenced more by tillage but also by the variety used. Plow tillage showed average values of 5.25 g, followed by chisel tillage of 4.70 g and disk harrow tillage of 2.75 g. The influence of

the varieties on the mass of kernels per plant leads to the highlighting of Orion and Flamenco varieties with 4.42 g and 4.38 g respectively. The varieties Burnas and Rodin had less than 4.05 g kernels weight per plant.

The influence of cultivated variety and tillage on the quality parameters in chickpea crop for the average of the research years 2023-2024, such as Thousand Kernel Weight (TKW), hectoliter mass (HM), protein and lipid content in kernels of chickpea crop are presented in Table 4.

Thousand Kernel Weight (TKW) registered the highest mean value in the plow tillage of 280.75 g, followed by the chisel tillage of 272.25 g and 263.90 g in the disc tillage, and the values differed widely among the varieties. In Orion the TKW was the highest at 324.12 g, thus justifying the higher yield production compared to Burnas which had the lowest TKW among the analyzed varieties of 239.95 g. The variety Flamenco succeeded after Orion with a TKW of 283.12 g and the variety Rodin with a low TKW of 241.93 g.

The average hectolitric mass (HM) was 74.40 kg and 74.65 kg, respectively for the plowing and discing tillage and 75.05 kg for the chiseling tillage. In terms of varieties, the average value was very low for Orion of 72.93 kg, for Flamenco it was 74.60 kg and for Rodin and Burnas the values were similar at 75.50 and 75.78 kg, respectively.

Kernel protein content was influenced by the tillage but also by the variety used. The average value was 24.72% in plow tillage, 24.35% in chisel tillage and significantly lower in disc harrow tillage of 23.28%. Among the varieties used, the Orion variety disclosed negatively with a low protein content of 23.21% in the kernels compared to the other varieties which had similar values of 24.23% for Burnas, 24.43% for Rodin and 24.59% for Flamenco.

The lipid content of the grains had similar values between the variants of 6.26% in the plow tillage, 6.24% in the chisel tillage and 6.18% in the disc harrow tillage and between the cultivated varieties ranged between 6.10% and 6.34%.

Table 4. Influence of cultivated variety and tillage on quality indicators in chickpea crop, average of research years 2023-2024

No. crt.	Tillage	Variety	TKW (g)	HM (kg)	Protein (%)	Lipid (%)
1	PLOWING	Burnas	245.30	75.95	25.21	6.54
2		Rodin	248.90	75.50	24.79	6.18
3		Flamenco	292.00	73.75	25.74	6.19
4		Orion	336.80	72.45	23,13	6.11
Plowing average			**280.75**	**74.40**	**24.72**	**6.26**
5	CHISEL	Burnas	241.50	76.00	23.79	6.23
6		Rodin	240.20	75.85	25.10	6.03
7		Flamenco	285.90	75.05	24.92	6.49
8		Orion	321.30	73.35	23.60	6.19
Chisel average			**272.25**	**75.05**	**24.35**	**6.24**
9	DISC HARROW	Burnas	233.05	75.40	23.70	6.25
10		Rodin	236.70	75.15	23.40	6.08
11		Flamenco	271.45	75.00	23.11	6.26
12		Orion	314.25	73.00	22.90	6.14
Disc harrow average			**263.90**	**74.65**	**23.28**	**6.18**
Varieties average		**Burnas**	**239.95**	**75.78**	**24.23**	**6.34**
		Rodin	**241.93**	**75.50**	**24.43**	**6.10**
		Flamenco	**283.12**	**74.60**	**24.59**	**6.31**
		Orion	**324.12**	**72.93**	**23.21**	**6.15**

CONCLUSIONS

The research conducted on the reddish preluviosolic soil type at Moara Domneasca has shown that for the chickpea crop, the varieties cultivated and the basic tillage are important elements for improving yield and quality parameters.

The obtained results clearly revealed differences between the chickpea varieties studied (Burnas, Rodin, Flameco and Orion), but especially between the tillage methods (plow at 25 cm

depth, chisel at 35 cm depth and disc harrow at 12 cm depth).

The highest yield was acquired by Orion variety in the experimental trial in which the basic work was the plowing tillage while the Flamenco variety had the highest protein and lipid content. Although the chickpea crop is tolerant to drought, due to the lack of rainfall unevenly distributed during the 2 years of trials as well as extremely high temperatures, the yield was low because of water deficiency.

The research results show that the chickpea yield varied greatly in terms of tillage and less in terms of cultivated varieties. The average yield of cultivated varieties for the variant using plowing tillage was 1566.0 kg/ha, a satisfactory yield considering the drought recorded during the trial.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

SOME *Asteraceae* SPECIES (*Asteraceae Martinov*) IN THE COLLECTION OF "ALEXANDRU CIUBOTARU" NATIONAL BOTANICAL GARDEN (INSTITUTE) AS POTENTIAL HONEY PLANTS

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Abstract

This article describes some plant species from the Asteraceae Martinov family, species with melliferous potential and multiple utility, from the collection of fodder, honey and energy plants of the "Alexandru Ciubotaru" National Botanical Garden, which includes a wide range of plants of various botanical families. Four species of non-traditional plants for the Republic of Moldova, belonging to the Asteraceae family, were selected as research subjects: Helianthus giganteus L., Silphium perfoliatum L.*, Cynara cardunculus* L.*, Inula helenium L. These plants are known for their high potential as fodder, honey, medicinal and energy plants. They are characterized by longevity, early onset of vegetation, long and staggered generative period, attractive flowers for a wide spectrum of pollinating and honey-bearing insects. The researched species can significantly contribute to the diversification of the honey base, as an additional source of nectar and pollen for useful entomofauna as well as the beekeeping industry of the Republic of Moldova.*

Key words*: Asteraceae Martinov, honey plants, development, flowering stages.*

INTRODUCTION

The *Asteraceae Martinov* (*Compositae* Adans.) family encompasses 18000-20000 species, included in around 1300 genera. Evolution has produced in many directions and the principal developmental lines are summarized by grouping related genera into tribes (Benson, 1957). The family was classified in several different ways over the years. For example, Cassini (1816-1830) in his papers, described 19 tribes (King & Dawson, 1975). Later, the classification was modified and simplified. Bentham (1873) described 13 tribes: Vernoniaeae, Eupatoriacea, Asteroideae, Inuloideae, Helianthoideae, Helenioideae, Anthemideae, Senecionideae, Calendulaceae, Arctotideae, Cynaroideae, Mutisiaeae and Cichoriaceae (Bentham & Hooker, 1873). Asteraceae is an advanced and botanically highly specialized family of mainly herbaceous plants. They are widely distributed in the tropics and warm temperate regions of South, South-East an East-Asia, Africa including Madagascar and Central - South America (Sharma, 2004.)

In the spontaneous flora of Bessarabia, the family includes 70 genera with 244 species (Ionita, 2022). It includes herbaceous plants, annuals, perennials, sub-shrubs or exceptionally - trees. The stem is simple or branched. The inflorescence - solitary or numerous pseudanthia (flower heads) arranged in compound spike-like, panicle or corymb inflorescences, at the base of the flowers often with bract-like scales or setae. The flower heads consist of ray flowers and disc flowers. The androecium is pentamerous. The fruits - monospermous achenes (Comanici et al., 2022; Ionita, 2022). The flowers are grouped in what is the most notable characteristic of the family, the capitulum, a pseudanthium (i.e. an inflorescence that mimics a single flower) (Classenn -Bockhoff, 1990). It is the largest family in the phylum Magnoliophyta, consisting of food, technical, ornamental, medicinal plants, but also some harmful weeds (Comanici et al., 2022).

The Asteraceae family includes many species of major economic importance. Some of its representatives are well known sources of raw materials such as vegetable oil, sunflower seeds, lettuce, sweeteners, coffee alternatives and herbal teas, others are medicinal plants - calendula, tansy, chamomile, wormwood, arnica, coltsfoot, echinacea, elecampane, milk

thistle, chicory etc., there are also weeds like dandelion, ragwort, groundsel etc., and ornamental plants such as dahlia, zinnia, cosmos, aster, sunflowers, marigolds, chrysanthemums etc., and some are important ornamental crops for the cut flower industry. (Singh et al., 2015; Funk et al., 2009). The researched species (*Helianthus giganteus* L., *Inula helenium* L., *Silphium perfoliatum* L., *Cynara cardunculus* L.) are described in specialized literature as valuable plants with multiple uses - medicinal, fodder, honey, food, decorative, energetic (Koltowski, 2005; Mantineo et al., 2009; Bolohan et al., 2013).

Some species of the Asteraceae family are known for their high melliferous potential, and the mobilization of non-traditional ones for the Republic of Moldova can significantly contribute to broadening the range of nectar-pollen sources, ensuring maintenance harvesting and varied production during the active season for beekeeping. Researching the structure of the honey plant base and the succession of blooms is of major importance for beekeepers as it could provide for a continuous source of pollen and nectar to be collected by bees. Melliferous flora can also contribute to wild bee abundance and to crop yields through enhanced floral diversity (Hevia et al., 2016).

The goal of our research was to conduct a study on the biological aspects of the development of plant species of the Asteraceae family with multiple utility, particularly as honey plants, in order to enrich the assortment of species and varieties useful for the national economy.

MATERIALS AND METHODS

The research was conducted within the "Alexandru Ciubotaru" National Botanical Garden (Institute), in the collection of forage, honey and energy plants, during the years 2022-2024, selecting for the current study plant species with melliferous potential, of the Asteraceae family, including less common plants for the Republic of Moldova. The selected species, according to their life form, are perennial plants with a long growing season, which can form a conveyor of flowers and food for the useful entomofauna, available from June to September. Finally, we selected as research subjects four species with high potential as honey, forage and energy crops: *Helianthus giganteus* L., *Inula helenium* L., *Silphium perfoliatum* L., *Cynara cardunculus* L. The germination capacity of the seeds of these species was determined in Petri dishes, seedlings were obtained in cell trays, under greenhouse conditions, and then the seedlings were transplanted outdoors, to the experimental sectors. The research was conducted according to the following techniques: Metodica izucenia fenologhii rastenii I rastitelinih soobscestv, Beideman, 1974.

Entomological surveys were carried out especially during the flowering stage (June - early September), at different times of the day, observations were made on the experimental sectors under various climatic conditions. The taxonomic affiliation of insects was determined using the species identification guidebook (Plaviliscicov, 1994).

The climatic conditions of the Republic of Moldova are characterized by mild winters and long, hot summers. The year 2023 was characterized by high temperature regime and a significant rainfall deficit during the August-October period. The year 2024 was also characterized by high temperature regime and rainfall deficit in the summer period, the annual amount of precipitation being 440-710 mm, and during the growing season it constituted 325-585 mm (meteo.md). According to the data from the Chisinau meteorological station, the average annual air temperature in 2024 was +13°C, that is, 3.2°C higher than normal, influencing the growth and development of agricultural crops.

RESULTS AND DISCUSSIONS

In the collection of forage, honey and energy plants of the "Alexandru Ciubotaru" National Botanical Garden (Institute) of MSU (NBGI), there is a wide variety of plant species with multiple uses, traditional and non-traditional species for the Republic of Moldova, belonging to various botanical families. Broadening the spectrum of honey plants, with new valuable species, will significantly contribute to enriching the raw material necessary for the maintenance and development of bee colonies, capitalizing on the honey base, with the ultimate objective being to increase the productivity of beekeeping. As the impact of climate change on

biodiversity exacerbates, annual species are the most affected; in this case, non-traditional perennial honey and forage crops, in addition to providing the necessary natural food for animals, will also contribute to improving the health and productivity of bee colonies by providing new sources of nectar and pollen.
The research conducted was based on the study of the biological peculiarities of plant growth and development under the climatic conditions of the Republic of Moldova.

Table 1. Seed germination capacity

Species	Germination capacity (%)	
	2023	2024
H. giganteus	12.00±1.16	7,55±0,23
I. helenium	66.33±18.84	24.33±0.39
S. perfoliatum	40.00±12.52	23.75±0.72
C. cardunculus	61.33±11.25	32.00±3.46

The repeated determination of the germination capacity of the seeds collected from the experimental sectors of the Botanical Garden (Table 1) demonstrated that the seeds harvested in 2024 had lower germination capacity as compared with those harvested in 2023, the reason being the weather conditions recorded in the summer months, being 3.0-4.4°C higher than normal. High temperatures were also recorded in September, the average exceeding the norm with 2.5-4.5°C and with low amounts of precipitation. These phenomena negatively influence the development and formation of the seeds as well as their viability. These phenomena negatively influence the development and formation of the seeds as well as their viability. *H. giganteus* seeds have low germination capacity (year 2023 - 12.00±1.16; year 2024 - 7.55±0.23) but, from viable seeds, healthy and hardy planting material can be obtained.
The current collection includes the following species of the genus *Helianthus* L.: *H. annuus, H. tuberosus, H. mollis* and *H. giganteus*, which are species with a high degree of genetic variability, thrive on soils with medium fertility, with a long flowering stage (30-35 days), being able to provide a food conveyor for insects from June to October (Cîrlig, 2020). *H. giganteus* L. 1753. (syn.: *H. subtuberosus* Britton 1901) (Figure 1A) is known by the common name giant sunflower, chromosome number: 2n = 34 (diploid); native to Northeast USA, Southeast Canada; it is an ornamental in Central Europe; sometimes it can escape (Lajos Balogh, 2008). It is a species with long growing season and long flowering stage.



A B

Figure 1. *H. giganteus*: A - mature plant in the flowering stage, B - honeybee visiting a flower

The plants grown in the NBGI, starting with the second year of vegetation, go through the complete development cycle every year. The generative phenological stages occur in the period June-September. In the budding stage (end of June), the plants reach 160-182 cm in height, developing by 16-18 first-order shoots per plant, these shoots, in turn, developing by 6-8 second-order lateral shoots. On a first-order shoot, there are on average 47-52 leaves. The length of the leaves is 15-17 cm, and the width is 4 cm. The full flowering stage occurs from middle to late July, varying slightly depending on the weather conditions. During this period, the plants are visited by large numbers of insects, and are particularly attractive to one of the most important species of honey bees - *Apis mellifera* (Figure 1B). A bee collects nectar from a flower for 4-8 seconds. Representatives of the species *Eristalis tenax* are present on a flower for about 14 seconds. Many ants, in search of food, participate in the pollination of flowers.
Inula helenium L. plants, the cultivar 'ILEANA', registered in 2021 in the Catalog of Plant Varieties of the Republic of Moldova, were also studied. They are perennial plants, with the growing season beginning in early to mid-April (older plants - in late April). The flowering phase is staggered, occurring in July-August (sometimes early September) and lasting about 25-30 days, depending on the age of the plants and climatic conditions. Since the generative

phenological stages are staggered, at the same time (July 26) there can be, for example, 55 floral buds and 26 flowers on a plant. On another specimen there were 46 floral buds, 32 flowers and 2-6 wilted flowers with fruits at the stage of early ripening. During this period, the stem at the base turned brown. The flowers are yellow, with a diameter of 5-8 cm, attractive to insects (Figure 2A). The flowers were visited by insects from the families Apidae, Syrphidae, Coccinellidae, which are recognized as effective pollinators.



A B

Figure 2. A - Flowers of *I. helenium*;
B - Flowers of *S. perfoliatum*

According to the research conducted at NBGI, it has been demonstrated that the ontogenetic cycle of cup plant (*S. perfoliatum* 'Vigor', registered in the Catalogue of Plant Varieties of the Republic of Moldova (p. 37) and patented at the State Agency for Intellectual Property (AGEPI) in 2016 (Teleuță & Țîței, 2016), includes 4 life periods: latent, pregenerative, generative, postgenerative and six age stages: seed, seedling, immature, juvenile, virginal and senile. During the seedling and immature stages, plant development highly depends on climatic conditions (Cîrlig, 2022). The germination capacity of seeds depends on several factors. Viable seeds were selected and sown in cell trays to obtain seedlings, which were later transplanted in the experimental sectors. In the first year of vegetation, the plants completed only the vegetative phenological stages. Starting with the second year of vegetation, the *S. perfoliatum* plants went through the entire ontogenetic cycle characteristic of this species. The beginning of the growing season was recorded in mid- March. Since it was researched as a honey plant, particular interest was paid to the generative phase. The flowering stage (Figure 2B) of cup plant lasts for 51-63 days, occurring in the period June-August or July-September, depending on the air temperature and soil humidity. The honey bee is present on the cup plant flowers and collects pollen from one flower for about 20 seconds, then moves on to another flower on the same plant. *S. perfoliatum* is known as a plant with high melliferous potential (150-450-560 kg honey/ha) (Koltowski, 2005), the nectar productivity can vary, between 205.2 and 611.6 kg/ha, being closely related to the quality of the soil and the amount of mineral fertilizers applied (Savin & Gudimova, 2019). Previous research conducted at NBGI demonstrated the presence, on *S. perfoliatum* plants, of 10 insect species belonging to 4 orders (Hymenoptera, Diptera, Coleoptera, Lepidoptera) and 6 families (Apidae, Halictidae, Syrphidae, Sarcophagidae, Cerambycidae, Satyridae) (Cîrlig, 2022).



A B

Figure 3. *C. cardunculus*: A - plants in the budding stage; B - flowers visited by insects

The species *Cynara cardunculus* L. (Figure 3), common names - cardoon or artichoke, under the climatic conditions of the Republic of Moldova, shows high tolerance to climatic factors, but temperatures below 0°C in spring can affect young plants, which easily recover once positive temperatures are recorded. In 2024, an experimental plot of *C. cardunculus* was obtained from seedlings started indoors, in cell trays. When the plants were 12-16 cm tall, they were transplanted to open ground, in late April, when the weather conditions were favorable for plants (the temperature recorded in April was +13°C…+15°C). At this stage, 5-6 leaves were developed, with a length of 12-14 cm and width of 3-4 cm. In the first year of vegetation, the plants developed only the rosette of basal leaves, which reached about 25-35 cm in height. In *C. cardunculus* plants, in the first

year of vegetation, the green leaves persisted till November.

Starting in the second year, the plants develop erect stems with alternate stem leaves and go through the full cycle of vegetative and generative phases. The flowering stage in artichokes occurs in July and lasts about 20 days. The number of inflorescences per plant at the beginning of July is on average 3.50±0.22. The inflorescence is a solitary terminal flower head, with purple-violet flowers. During this period, the flowers are abundantly visited by insects, the plants being described in the specialized literature as potential honey plants, with a productivity of 150-400 kg/honey/ha (https://www.apiterapie.ro/). The production potential of honey plants differs from year to year and directly depends on the weather conditions recorded in that year.

The phenological spectrum was determined by field surveys made at 2-4 day intervals. During the growing season, plants manage to go through the entire cycle of vegetative and generative phenological stages. In the current study, the generative phenological stages were highlighted - budding, flowering and fruiting (Table 2). The beginning of the growing season varies from one species to another and takes place between late March, for *C. cardunculus* (17.03.2023/20.03.2024) and late April, for *H. giganteus* (20.04). The full flowering stage was recorded in mid-July, but the periods of fruit formation and development were more diverse, depending on the duration of the flowering stage of each species. In the species *H. giganteus* and *S. perfoliatum* the fruit development stage was recorded in early to mid-September. The end of the growing season (beginning of dormancy), regardless of the species and age of the plants, took place in autumn, in October-November, when freezing temperatures were recorded at night (-2°C-4°C). The dry leaves fell from the shoot, only dry and browned seeds remained on the stems.

Table 2. The phenological spectrum of the studied species

Year	2022				2023				2024			
Species	S	B	Fl	Fr	S	B	Fl	Fr	S	B	Fl	Fr
H. giganteus	20.04	07.09	28.09	15.10	08.04	25.06	20.07	18.09	4.04	28.06	28.07	13.09
I. helenium	12.04	08.06	14.07	06.08	11.04	12.06	18.07	15.08	10.04	12.06	06.07	16.07
S. perfoliatum	04.04	22.06	11.07	04.09	03.04	22.06	18.07	06.09	02.04	21.06	08.07	02.09
C. cardunculus	01.04	13.06	20.07	16.08	17.03	20.06	23.07	18.08	20.03	16.06	15.07	20.08

Note: S - sprouting; B - budding; Fl - flowering; Fr - fruit development.

The flowering stage of the researched species is long, staggered, they can form a food conveyor for useful entomofauna during the months of July-September, which also demonstrates the presence of a wide spectrum of insects in the flowering stage, insects that actively participate in the pollination process. The flowers are visited by insects throughout the day, from early morning until evening. Representatives of the Apidae family, which includes bumblebees, solitary bees, stingless bees and honey bees, are present in large numbers on flowers. There are approximately 700 species of bees in Central Europe, and the honey bee is the only pollinator species that lives in perennial societies (Kunast et al, 2023). From an ecological point of view, bees are considered biological resources that play an essential role in the perpetuation and survival of numerous plant species on Earth, being a true biometer of ecological balance (Lazăr & Vornicul, 2007).

Most bees depend on a diversity of plant species, requiring a constant supply of nectar and pollen. In addition to Apidae species, recognized as the most basic pollinators (*Apis mellifera, Bombus* species), individuals of the *Eristalis tenax* species, Coccinelide species, and ants were detected on the researched plants. The species *Cetonia aurata* and *Epicometis hirta* are often found on artichoke flowers. The insects are active throughout the day, but between 12:00 and 14:00, their number is lower on the plants, the air temperature during this period reaching its maximum in July and August.

CONCLUSIONS

The results of the research carried out at the "Alexandru Ciubotaru" National Botanical Garden (Institute) on plants from the Asteraceae family (*Helianthus giganteus* L., *Inula helenium* L., *Silphium perfoliatum* L., *Cynara*

cardunculus L.) have demonstrated the melliferous potential of the studied species, which would help broadening the spectrum of honey plants available in the Republic of Moldova, for the possibility of organizing an uninterrupted honey conveyor for a long period. Phenological and entomofaunistic research has demonstrated that the plants studied have honey value, through their long flowering period (*S. perfoliatum* 51-63 days, *H. giganteus* 30-35 days) as well as the presence in large numbers of *Apis mellifera* specimens on flowers throughout the flowering phase of the plants. The researched species can serve as alternative honey plants for the Republic of Moldova, by extending the honey season to August-September. Besides, they are valuable plants to support the useful entomofauna, along with other species of traditional honey plants. These species are characterized by long growing season and staggered flowering stage, which corresponds to the period of June-September. The germination capacity of seeds varies from year to year, being influenced by temperature and the amount of humidity. All the plants come out of dormancy in early spring, in 2024 - *C. cardunculus* on 20.03; *S. perfoliatum* - 02.04; *H. giganteus* 04.04 and *I. helenium* - 10.04.
The plants provide food for a wide range of insects, especially representatives of the Apidae family: *Apis mellifera*, *Bombus* species, known as honey producing insects and active pollinators. The honey bee collects pollen and nectar from a *H. giganteus* flower for 4-8 seconds, from a *S. perfoliatum* flower for about 20 seconds.

ACKNOWLEDGEMENTS

This research has been supported by the subprogram no. 01.01.02 "*Identification of valuable forms of plant resources with multiple uses for the circular economy*".

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

EVALUATION OF QUANTITATIVE AND QUALITATIVE INDICATORS IN MAIZE HYBRIDS (*Zea mays* L.) CULTIVATED IN NORTH-EASTERN OF BULGARIA

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Abstract

Maize (Zea mays L.) is one of the most produced cereal crops on a global scale. The field experiment was held in the experimental of the selected area in region Razgrad (North-Eastern Bulgaria) during the period 2021-2023. The randomized complete block design with 4 replications and 25 m^2 plot size was applied with five maize hybrids - P9415 (FAO 360); P9889 (FAO 420); P9903 (FAO 420); KAPITOLIS (FAO 380) and INTELIGENS (FAO 420). The purpose of the study was to establish the productivity and quality of some maize hybrids, grown in North-Eastern Bulgarian region. The following characteristics were reported: grain yield; cob length; number of rows per cob; number of grains per row; mass of grain per cob; 1000 grains weight; test weight and crude protein. The analysis of the results showed that the highest grain yield was obtained from P9889 hybrid due to the higher values of yield structural elements, while the highest values of the crude protein were reported by the hybrid INTELIGENS. The values of the indicators 1000 grains weight and test weight were highest by the hybrid KAPITOLIS (313 g and 74.2 kg, respectively).

Key words*: maize, hybrids, grain yield, quality, protein.*

INTRODUCTION

Maize (*Zea mays* L.) is one of the main food and technical crops in the world's grain production. It is used for a great number of purposes – gran, fodder, technical, etc. The biological potential of the crop is determined by hybrid, specific meteorological conditions during the respective economic year and the technology of growing the plants (Ali et al., 2020; Dimitrova et al., 2013; Kirchev, 2016; Komitov et al., 2022; Kuneva et al., 2015; Manilov and Manhart, 2024; Kirchev, 2016; Yankov et al., 2014; Yan et al., 2021). The hybrid with its specific genetic potential is the most dynamic factor in production (Horablaga et al., 2023; Hussain et al., 2011; Katsenios et al., 2021; Mosisa et al., 2023). Seed producers use breeding to develop hybrids that are more tolerant to climate changes and can withstand such high temperatures and droughts (Duvick, 2005; Nagy, 2004; Șuteu et al., 2013). The growing of high-quality hybrids not only improves the grain yield and its quality but also increases the income per hectare (Dimitrova et al., 2021; Gul et al., 2020; Yousif et al., 2001; Shahini et al., 2023).

Even though there was a tendency to select the high-yield hybrids, the need for overall stability and dependability favors the selection of hybrids with stress tolerance. The main focus of the new hybrids now is to aim for a high and stable yield in both favorable and unfavorable growing conditions (Duvick, 2005; Katsenios et al., 2021).

The agroecological and climatic conditions in the separate regions of the country influence the development and productivity of maize (Delibaltova et al., 2019; Ruswandi et al., 2021; Stoyanov and Kuneva, 2024).

The right choice of hybrids and the proper regional distribution, as well as their growing by strictly following the agrotechnical practices, are of vital importance for the yield amounts and the quality of the produce obtained (Dallev and Ivanov, 2015; Delibaltova, 2018; Dimitrova et al., 2019; Mandić et al., 2020; Mitkov et al., 2018; Yanev, 2023; Zivkov and Matev, 2005). That necessitates the constant introduction of new maize hybrids that are the most suitable and efficient for the separate microregions of the country.

The aim of the investigation was to establish the productivity and quality of five maize

hybrids for grain, grown in the North-Eastern Bulgarian region.

MATERIALS AND METHODS

The field experiment was held in the experimental of the selected area in region Razgrad (North-Eastern Bulgaria) during the period 2021-2023. The randomized complete block design with 4 replications and 25 m^2 plot size was applied, after previous crop winter wheat. Five maize hybrids - P9415 (FAO 360); P9889 (FAO 420); P9903 (FAO 420); KAPITOLIS (FAO 380) and INTELIGENS (FAO 420) were included in the study. The experiment was carried out following the adopted cultivation technology under non-irrigated conditions. Soil cultivation included ploughing in of the stubble in August and plowing at a depth of 28-30 cm in October, pre-sowing cultivation with harrowing was performed twice in March and April. Fertilization was performed in autumn before deep ploughing, with 80 kg/ha active substance phosphorus and 10 kg/ha active substance potassium and before sowing with 180 kg/ha active substance nitrogen. The sowing was performed in the third decade of April at the spacing between the rows of 70 cm and a seed rate of 65000 germinating seeds per ha at a depth of 6-8 cm. Weed control was achieved by treatment with the herbicide Principal plus - 440 g/ha (920 g/ha nicosulfuron, 5500 g/ha dicamba, 230 g/ha rimsulfuron) applied during the maize vegetation (after formation of 3-5 leaves). During the vegetation the inter rows were cultivated twice. All the steps of the adopted maize cultivation technology were respected.

The following characteristics were reported: cob length; number of rows per cob; number of grains per row; mass of grain per cob; grain yield; test weight; 1000 grains weight and crude protein.

The experimental data were processed by the method of dispersion and correlation analyses and the differences between the variants were determined by means of the Dunkan's Multiple Range Test.

The climatic conditions determining maize growth, development and productivity were temperatures and precipitation, their combination and distribution during the vegetative period. The analysis of these factors showed that the values of the average monthly temperatures during the years of study did not differ significantly from those of the multi-year period and satisfied completely the requirements of the maize towards heat from the period of sowing to the period of ripening (Figure 1).

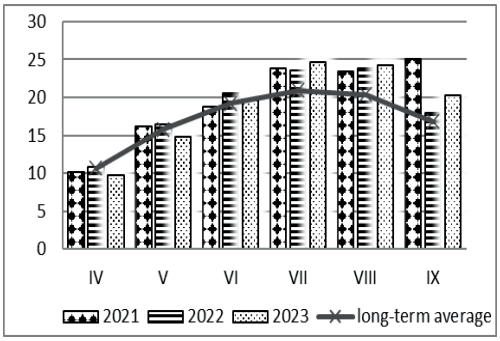


Figure 1. Average monthly air temperature, ^0C

Significant differences were observed in the quantity of precipitation during the individual agricultural years (Figure 2).

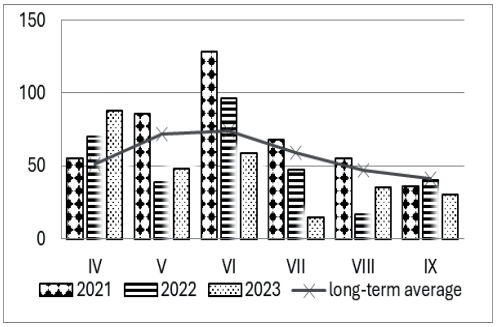


Figure 2. Precipitation, mm

In the first year of the experiment (2021), the amount of precipitation in the April-September period was 428 mm, which is with 84 mm more than the multiannual period (344.0 mm). The good moisture supply and the uniform distribution of rainfall from the beginning of the vegetation to the beginning of ripening had a beneficial effect on the growth and development of maize plants.

In 2023, the amount of rainfall during the vegetation was with 69 mm less than the multi-year period and with 153 mm less than in 2021.

The significant amount of precipitation in the period April - June (195 mm) was followed by a drought period. The lack of precipitation, combined with high temperatures, negatively affected the growth processes and productive capabilities of the maize plants.
The second year of the study the amount of precipitation during the of maize vegetation was 309 mm, which is 35 mm lower than the multiannual period. That determined the 2022 experimental year as less favorable for the productivity of maize compared to 2021.

RESULTS AND DISCUSSIONS

The impact of both factors - hybrid and year on the grain yield, as well as on the other qualitative components are presented in Table 1. Data showed that both by years and in average for the experimental period hybrid, P9889 surpassed in grain yield all the other hybrids included in the study. The higher grain yield of the hybrid was due to the higher values of its structural elements.
The highest grain yields were obtained in the favorable for maize 2021 year when the temperature values and the precipitation sum fully met the plant requirements for warmth and moisture throughout the whole vegetation period. The yields obtained reached up to 8760 kg/ha. The hybrid P9889 surpassed the hybrids P9903, INTELIGENS, KAPITOLIS and P9414 by 1.4%, 10.4%, 11.8% and 14.9%, respecttively, the differences being statistically significant.
In the 2022 experimental year the grain yields obtained varied from 6483 kg/ha to 7226 kg/ha, i.e. they were by 20.5% lower in average in comparison with the previous year. The differences between the studied hybrids were mathematically significant. The lowest yields were realized by hybrid P9415 - 6483 kg/ha.
In the third year of the study (2023) the meteorological conditions during the hybrid vegetation were extremely unfavourable and the plants were not able to attain their biological potential. The grain yields obtained were within the limits of 5225 to 5820 kg/ha. Statistically proven, the lowest ones were those of hybrid P9415 and the highest - of P9889. The results of the hybrids P9903 and INTELIGENS had quite close values and they were statistically insignificant.
Average for the period of examination of the tested hybrids the highest yield is obtained of P9889 - 7269 kg/ha he is superior to 2.3, 6.8 and 9.0% hybrids P9903, INTELIGENS and KAPITOLIS, and the lowest yield is realized to P9415 (6443 kg/ha). The dispersion analysis shows a strong statistically proven influence of both the tested hybrids and the years with their specific climatic conditions on indicator “grain yield”. An interaction between Hybrid and Year was proven.
Differences in the climatic conditions during the years of the experiment are one of the reasons for the formation of cobs with different length. In the first experimental year (2021), the cob length varied from 19.5 cm in hybrid P9415 to 21.4 cm in - P9889, while in 2023 the values of this indicator were significantly lower (on average by 38%) and ranged from 14.0 to 15.6 cm, and in 2022 - from 17.2 to 19.1 cm. On average for the study period, the cob length of hybrid P9889 was 18.7 cm and surpassed hybrids P9903 and INTELIGENS by 6.2%, KAPITOLIS by 8.7%, and P9415 by 10.6%.
The analysis of variance (Anova) shows the impact of the factors hybrid and year, together with their interaction, on cob length indicator and the results indicate a statistically significant influence of the examined factors and significant effect of their interaction.
Genetically controlling factor is the number of rows per cob but environmental conditions may also influence on this indicator. The more number of rows per cob results in more grain yield (Tahir et al., 2008). Maximum number of rows per cob was found in P9889
hybrid as well as by years and in average for the experimental period hybrid. The dispersion analysis about the effect of the factors Hybrid and Year, as well as their interaction, on the number of rows per cob, shows a significant influence of the factors on the changes of the characteristic and statistically insignificant effect of the interaction between them.
The studied hybrids differed significantly in the number of grains per row. The lowest values of that indicator were reported in 2023 when the climatic conditions were less favorable for the growth and development of maize compared to 2021 and 2022. Mathematical processing of data showed that the differences between all the studied hybrids were significant. Out of all

the investigated hybrids the smallest number of grains per the row, in average for the three years, was established in hybrid P9415 - 30.5 and it fell behind the hybrids INTELIGENS, KAPITOLIS and P9903 by 6.9, 8,8 and 11,1%, respectively, the biggest values being formed by hybrid P9889 (37.0).

Table1. Quantitative indices in maize hybrids

Variable		Grain Yield, kg/ha	Cob length cm	Number of rows per cob	Number of grains per row	Mass of grain per cob, g
Years	2021	8159 ^{a}	20.2 ^{a}	15.0 ^{a}	36.8 ^{a}	164.2 ^{a}
(A)	2022	6771 ^{b}	18.0 ^{b}	14.2 ^{b}	34.6 ^{b}	140.5 ^{b}
	2023	5643 ^{c}	14.6 ^{c}	13.4 ^{c}	28.9 ^{c}	94.3 ^{c}
Hybrid	P9415	6443	16.9	13.3	30.5	120.7
(B)	P9889	7269	18.7	15.3	37.0	144.2
	P9903	7103	17.6	14.3	33.9	137.6
	KAPITOLIS	6666	17.2	14.0	33.2	129.7
	INTELIGENS	6808	17.6	14.1	32.6	132.7
2021	P9415	7622 ^{e}	19.5 ^{c}	14.0 ^{c}	34.0 ^{d}	157.2 ^{d}
	P9889	8760 ^{a}	21.4 ^{a}	16.4 ^{a}	42.0 ^{a}	174.8 ^{a}
	P9903	8643 ^{b}	20.2 ^{b}	15.2 ^{b}	37.2 ^{b}	167.3 ^{b}
	KAPITOLIS	7837 ^{d}	19.7 ^{c}	14.3 ^{c}	35.2 ^{c}	160.7 ^{c}
	INTELIGENS	7934 ^{c}	20.1 ^{b}	15.0 ^{b}	35.6 ^{c}	161.0 ^{c}
2022	P9415	6483 ^{e}	17.2 ^{c}	13.4 ^{c}	30.0 ^{c}	120.7 ^{e}
	P9889	7226 ^{a}	19.1 ^{a}	15.0 ^{a}	38.0 ^{a}	160.0 ^{a}
	P9903	6895 ^{b}	18.0 ^{b}	14.4 ^{b}	36.0 ^{b}	149.5 ^{b}
	KAPITOLIS	6520 ^{d}	17.4 ^{c}	14.0 ^{bc}	35.0 ^{c}	132.3 ^{d}
	INTELIGENS	6732 ^{c}	18.4 ^{b}	14.1 ^{b}	34.0 ^{d}	140.0 ^{c}
2023	P9415	5225 ^{d}	14.0 ^{c}	12.6 ^{c}	27.5 ^{d}	84.3 ^{d}
	P9889	5820 ^{a}	15.6 ^{a}	14.0 ^{a}	31.0 ^{a}	97.8 ^{a}
	P9903	5770 ^{b}	14.7 ^{b}	13.4 ^{b}	28.4 ^{c}	96.0 ^{c}
	KAPITOLIS	5642 ^{c}	14.6 ^{b}	13.6 ^{b}	29.5 ^{b}	96.2 ^{c}
	INTELIGENS	5760 ^{b}	14.3 ^{bc}	13.2 ^{b}	28.3 ^{c}	97.0 ^{b}
Anova	A	**	**	*	**	**
	B	**	*	*	*	**
	AB	*	*	n.s	*	*

Means within columns followed by different lowercase letters are significantly different (P<0.05) according to the LSD test F-test significant at P<0.05; ** F-test significant at P<0.01; ns non-significant.

The results of the dispersion analysis about the effect of the factors Hybrid and Year, as well as their interaction, on the indicator “number of grains per row”, show a statistically significant effect of the studied factors and significant of their interaction.

An important indicator on which the yield of maize depends is the mass of grains per cob and the factors influencing the values of this characteristic are the technology of cultivation, the weather conditions, as well as the genetics of the hybrid (Ali et al., 2020; Devasree et al., 2020).

Differences in the climatic conditions during the years of the experiment are one of the reasons for the formation of grain with different mass. The largest mass of grains in a cob was observed in the firs year, because of the optimal weather conditions. With a value of 174.8 g hybrid P9889 distinguished significantly from the other hybrids. The differences among the studied hybrids were statistically significant. In the second year, mass of the grain per cob varied from 120.7 g by hybrid P9415 to 160.0 g by hybrid P9889 and the differences were proven. In line with the observations for the grain yield, the lowest values of mass of the grain per cob are reported in the third year, when the conditions were not so favourable and varied - from 83.4 for P9414 hybrid to 97.8 g - for P9889 hybrid. The values of P9903 were very closed to those of KAPITOLIS and the differences between both hybrids were unproven.

On average for the study period, P9889 hybrid was realized the mass of grains per cob of 144.2 g and surpassed hybrids P9903, INTELIGENS, KAPITOLIS and P9415 by 4.8,

8.7, 11.2 and 19.5%, respectively. The analysis of variance (ANOVA) shows a strong statistically proven influence on both the tested Hybrids (B) and the years with their specific climatic conditions (A) on the indicator mass of grain per cob. An interaction between Hybrid and Year was proven.

Table 2. Qualitative components in maize hybrids

Variable		1000 seed weight (g)	Test weight (kg)	Crude protein (%)
Years	2021	349.4 [a]	73.6 [a]	9.6 [c]
(A)	2022	304.0 [b]	71.5 [b]	10.6 [b]
	2023	238.4 [c]	68.8 [c]	11.4 [a]
Hybrid	P9415	292	70.3	10.6
(B)	P9889	300	70.8	9.9
	P9903	302	71.0	10.4
	KAPITOLIS	313	74.2	10.5
	INTELIGENS	280	70.1	11.3
2021	P9415	345 [d]	73.5 [bc]	9.6 [b]
	P9889	352 [c]	72.4 [c]	9.4 [bc]
	P9903	360 [b]	73.2 [bc]	9.5 [b]
	KAPITOLIS	368 [a]	76.0 [a]	9.5 [b]
	INTELIGENS	322 [c]	72.8 [c]	10.2 [a]
2022	P9415	300 [c]	70.4 [b]	10.7 [b]
	P9889	310 [b]	71.6 [b]	9.7 [c]
	P9903	307 [b]	71.0 [b]	10.6 [b]
	KAPITOLIS	315 [a]	75.4 [a]	10.7 [b]
	INTELIGENS	288 [d]	69.3 [c]	11.4 [a]
2023	P9415	231 [c]	67.1 [c]	11.4 [b]
	P9889	237 [b]	68.5 [b]	10.8 [c]
	P9903	240 [b]	68.9 [b]	11.2 [b]
	KAPITOLIS	255 [a]	71.2 [a]	11.3 [b]
	INTELIGENS	229 [c]	68.1 [b]	12.2 [a]
Anova	A	**	*	**
	B	*	*	*
	AB	*	n.s	*

*Means within columns followed by different lowercase letters are significantly different (P<0.05) according to the LSD test
*F-test significant at P<0.05; ** F-test significant at P<0.01; n.s non-significant

The impact of both variable hybrid and year on the qualitative components is presented in Table 2. The 1000 grains weight are influenced by environmental conditions, cultivation technology and genetics of the hybrids (Ali et al., 2020; Georgieva et al., 2023) The largest mass per 1000 grains was observed in the first year, because of the optimal weather conditions. With a value of 368 g hybrid KAPITOLIS distinguished significantly from the other hybrids. The lowest values of that characteristic were reported in hybrid INTELIGENS - 322 g. The differences between the hybrids were statistically significant. In the economic year 2022 the 1000 grain weights varied from 288 to 315 g, i.e. by 11.8 to 16.8% lower than 2021. The lowest values of that characteristic (within 229 to 255 g) was obtained in the third year.

During the period of study (2021-2023) KAPITOLIS hybrid realized the1000 grains weight of 313 g in average and it surpassed the hybrids P9903, P9889, P9415 and INTELIGENS by 11 g, 13 g, 21 g and 33 g, respectively.

The dispersion analysis about the effect of the factors Hybrid and Year, as well as their interaction, on the 1000 grains weight shows a significant influence of the factors on the changes of indices and statistically significant effect of the interaction between them.

The test weight is an indicator of the commercial quality of grains, and it plays an important role in determining the sale price. The lowest values of indicator were established in the last experimental year (2023), in the tested hybrids varied from 68.1 kg (INTELIGENS) to 71.2 kg (KAPITOLIS). It showed that the low amount of rainfall during the vegetation has a negative influence on grain formation. The highest values of test weight were established in the first year of the study

(2021), from 72.4 to 76.0 kg. During the three-year period, values over 74.2 kg were documented for the hybrid KAPITOLIS. The lowest average value of test weight for INTELIGENS hybrid was observed at 70.1 kg. The analysis of variance (ANOVA) shows the impact of the factors hybrid and year, together with their interaction, on the test weight indicator and the results indicate a statistically significant influence of the examined factors and an insignificant effect of their interaction.

Unlike the other studied indicators, which were higher in the year with the highest and evenly distributed rainfall, the crude protein is favored by dry, hot weather, as well as by the lesser amount of precipitation during the grain-ripening stage. Due to dilution the increased amount of precipitation resulted in higher grain yields, but lower crude protein values (Georgieva et al., 2023; Mahmood et al., 2019). The highest protein content in grain was established in 2023 and varied from 10.8% in P9889 to 12.2% in INTELIGENS. In the other years of the experiment, the values of that characteristic were by 9.7 to 11.4 % and by 9.4 to 10,2% in 2022 and 2021, respectively. For the tested period hybrid INTELIGENS was distinguished with the highest values of protein content 11.4 % and surpassed the hybrids P9415, KAPITOLIS, P9903, P9415 and P9889 by 6.6%, 7.6%, 8.9% and 14.1%, respectively. The ANOVA variance analysis indicates a substantial impact of the two parameters, hybrid and year, on crude protein content. Their interaction has also been substantiated.

The analysis of correlation among the between the qualitative and quantitative parameters is shown in Table 3. The results of correlation evidenced that grain yield strong and positive associations (r>0.9) with cob length, mass of grains per cob and 1000 grains weight.

Table 3. Values of the coefficient of corelation

	Grain yield	Cob length	Number of rows per cob	Number of grains per row	Mass of grains per cob	1000 grains weight	Test weight	Crude protein
Grain yield	1							
Cob length	**0.958**	1						
Number of rows per cob	0.644	0.639	1					
Number of grains per row	0.848	0.877	0.673	1				
Mass of grains per cob	**0.950**	**0.970**	0.629	0.883	1			
1000 grains weight	**0.914**	**0.915**	0.540	0.793	**0.921**	1		
Test weight	0.581	0.609	0.341	0.523	0.631	0.722	1	
Crude protein	-0.845	-0.834	-0.501	-0.717	-0.825	-0.845	-0.567	1

*significance level alpha = 0.05

Strong positive values were reported for cob length and mass of grains per cob; 1000 grains weight and mass of grains per cob; cob length and 1000 grains weight. Negative correlation was observed between the crude protein and all other the qualitative and quantitative indicators. The same tendency was reported also from Georgieva et al. (2023) and Werle et al. (2014).

CONCLUSIONS

The qualitative and quantitative indices of tested maize hybrids in North-Eastern of Bulgaria largely determined by the meteorological conditions of the year and, above all, by the amount and the distribution of the precipitation as well as by genotype. The results of this study suggest the highest grain yield was obtained from P9889 hybrid due to the higher values of yield structural elements. Hybrid INTELIGENS distinguished with the highest values of the crude protein 11.3%. Grain yield and the crude protein content were negatively related. The values of the indicators 1000 grains weight and test weight were highest by the hybrid KAPITOLIS.

Out of the studied maize hybrids for the region of Razgrad, it is recommended to cultivate hybrid P9889 where the productivity and the quality are balanced under the contrast conditions of the studied years.

ACKNOWLEDGEMENTS

This experiment was carried out with the support of Project 17-12 at The Centre of Research, Technology Transfer and Protection of Intellectual Property Rights at the Agricultural University of Plovdiv, Bulgaria

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

SCREENING OF BARBED GOATGRASS (*Aegilops triuncialis* L.) FOR NaCl SALINITY STRESS AT GERMINATION AND EARLY STAGES OF PLANT GROWTHT

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Abstract

The objective of this study was to evaluate the genotypes of barbed goatgrass (Aegilops triuncialis L.) for tolerance to NaCl salinity stress at germination and the early stages of plant growth. Seeds from five populations of Aegilops triuncialis L. were collected from disparate regions of Bulgaria and subjected to salt stress at six distinct NaCl concentrations (50, 100, 150, 200, 250 and 300 mM). The genotypes exhibited considerable variation for the germination and seedling characteristics examined under both control and salinity-stress conditions. The highest value of the relative injury rate was recorded when seeds were treated with 300 mM NaCl. The application of increasing concentrations of the NaCl prolonged the mean germination time and suppressed the studied germination and seedling traits and had a greater inhibitory effect on shoot growth compared to root growth. Genotype BGR43665 was identified as the most tolerant to salinity stress, when exposed to increasing concentrations of sodium chloride ranging from 50 to 250 mM, on the base of the indices values for Mean productivity (MP), Geometric mean productivity (GMP), Harmonic mean (HM), Stress tolerance index (STI), Yield index (YI), Yield stability index (YSI), Relative stress index (RSI), calculated on the based of shoot dry mass. The average of the summary ranks (ASR) also identified BGR43665 as the best genotype.

Key words*: Aegilops triuncialis L., germination, early growing stage, salinity, tolerance.*

INTRODUCTION

Elevated salinization is identified as a significant detrimental environmental stress, particularly in desert and semi-desert climates, leading to substantial yield losses for a wide range of domesticated plants worldwide. More than 20% of the irrigated area on Earth is damaged by salt, with an increasing trend due to climate change and overexploitation of groundwater resources (Wu et al., 2017; Alam et al., 2021; Pour-Aboughadareh et al., 2023; Fatema et al., 2024; Kotula et al., 2024; Zhou et al., 2024). Salt stress affects almost all plant components and leads to a significant reduction in biomass production and ultimately yield (Seeda et al., 2022). Plants are affected by salt stress in two ways: osmotic stress and ionic stress. Osmotic stress is caused by high salt content in the soil, which reduces water potential and limits the amount of water plants can take up. It leads to dehydration and various physiological responses of plants. Ionic stress is associated with the accumulation of toxic sodium (Na+) and chloride (Cl-) ions in plant tissues, leading to disruption of ionic homeostasis and displacement of important potassium (K+) ions (Waheed et al., 2024). Plants exhibit adverse responses to salt stress, which can impede nutrient uptake, disrupt ion balance, and hinder growth (Bano et al., 2023). The capacity of plants to withstand excessive salt levels in their environment is variable, and they are typically classified into four distinct categories: salt-sensitive, moderately salt-sensitive, moderately salt-tolerant, and salt-tolerant (Zhou et al., 2024).

It is widely acknowledged that the germination of seeds and the subsequent growth of seedlings represent the most critical yet vulnerable phases in the life cycle of plants. Consequently, the focus of salinity research has been predominantly on these two stages (Ehtaiwwesh & Emsahel, 2020; Dehnavi et al., 2020; Mohammadi et al., 2023; Irik & Bikmaz, 2024). Increasing salinity concentration has

been found to reduce osmotic potential, thereby limiting germination rate, emergence rate and root development. The decrease in total seed germination under salt stress conditions is due to the occurrence of metabolic disturbances expressed in an increase in phenolic compounds. It has been suggested that the decline in total germination percentage is associated with a reduction in water absorption into seeds (Podder et al., 2020). Dehnavi et al. (2020, 2024) noted that, salt stress tolerance at the germination and seedling emergence stages has been shown to determine better plant establishment in saline soils. Ehtaiwesh et al. (2024) observed that the identification of salinity-tolerant crops and their diverse genotypes with the potential to withstand salinity is of paramount importance, given the trends and future demographic projections. Furthermore, the breeding of important crops with the potential to develop salinity-tolerant genotypes can facilitate the efficient use of salt-affected land and saline water resources.

The objective of the present study was to evaluate the genotypes of barbed goatgrass (*Aegilops triuncialis* L.) with regard to their tolerance to NaCl salinity stress during the processes of germination and the early stages of plant growth.

MATERIALS AND METHODS

In this investigation, five barbed goatgrass (*Aegilops triuncialis* L.) accessions collected from different locations across the Bulgarian territory were evaluated for their response to NaCl salinity stress under laboratory conditions (Table 1). The effect of six different salt concentrations (50 mM, 100 mM, 150 mM, 200 mM, 250 mM, 300 mM) on seed germination and early seedling characteristics were investigated. Deionized water was used as control. Two replicates of 25 seeds of each treatment were plated for germination on filter paper with 20 ml of the test solutions. The paper with the seeds was placed in plastic bags to keep them moist. Before starting the germination test, the samples were placed for one week on refrigeration at 4°C. Seed germination was at 20±1°C for 8 days. The paper was changed every two days to prevent salt accumulation. Seeds were considered germinated when the germ was at least 2 mm long. The number of germinated seeds was recorded each day. The following germination characteristics were recorded- germination percentage (G, %), coefficient of velocity of germination (CVG), germination rate index (GRI, % day^{-1}), mean germination time (MGT), and relative injury rate (RIR). CVG was calculated using Kader & Jutzi (2004), GRI and MGT using Kader (2005), and RIR using Li (2008). On day 8, 10 seedlings were randomly selected from each treatment to measure shoot and root length and fresh weight. Dry weight of the shoot and the root were recorded after drying in an oven at 80°C for 24 hours. Seedling height reduction was determined using the method described by Islam & Karim (2010).

To characterize the response of the genotypes at different salinities and to select the most tolerant genotype, the following tolerance and susceptibility indices were calculated using the user-friendly online software iPASTIC: Tolerance Index (TOL), Relative Stress Index (RSI), Mean Productivity (MP), Harmonic Mean (HM), Yield Stability Index (YSI), Geometric Mean Productivity (GMP), Stress Susceptibility Index (SSI), Stress Tolerance Index (STI) and Yield Index (YI) (Pour-Aboughadareh et al., 2018).

Analysis of variance (ANOVA) was used to test the effects of salt concentration, genotype and their interaction on the measured traits. Comparisons of means were made using the Duncan´s multiple range test at the probability level ($p < 0.05$) using SPSS software.

Table 1. Passport information
for the accessions included in the study

Accession number	Species	Origin	Geographical coordinates		
			Latitude	Longitude	Altitude
BGR43679	*Aegilops triuncialis* L.	BGR	41°51'20''N	26°04'46''N	74
BGR43540	*Aegilops triuncialis* L.	BGR	41°52'22''N	26°00'15''N	88
BGR43669	*Aegilops triuncialis* L.	BGR	41°39'29''N	25°38'60''N	342
BGR43307	*Aegilops triuncialis* L.	BGR	41°37'31''N	25°40'30''N	166
BGR43665	*Aegilops triuncialis* L.	BGR	41°42'45''N	25°36'20''N	342

RESULTS AND DISCUSSIONS

A two-way ANOVA analysis (Tables 2, 3) showed a significant individual effect of genotype, concentration (dose) and their interaction on the studied germination and seedling characteristics. The study revealed that salt concentration exerted the most substantial effect on the majority of the examined

parameters, with the exception of the germination percentage, where genotype and the applied dose exhibited nearly equivalent impacts on trait expression.

Table 2. Two-way ANOVA on the effect of genotype, concentration and their interaction on the germination characteristic of *Aegilops triuncialis* L

Source of Variation	*df*	*MS*	*Sign.*	η^2, %
CVG				
Genotype	4	515.21	0.00	7.86
Concentration	6	3276.18	0.00	**74.93**
Interaction	24	122.77	0.00	11.23
GRI				
Genotype	4	2505.93	0.00	22.90
Concentration	6	4795.16	0.00	**65.72**
Interaction	24	108.11	0.00	5.93
MGT				
Genotype	4	7.08	0.00	11.73
Concentration	6	20.62	0.00	**51.24**
Interaction	24	3.27	0.00	32.47
G				
Genotype	4	8126.79	0.00	**40.87**
Concentration	6	5912.14	0.00	**44.60**
Interaction	24	313.04	0.00	9.45

G-germination percentage, CVG-coefficient of velocity of germination, GRI-germination rate index, MGT-mean germination time, η2-degree of influence of the factors (%), df = degrees of freedom.

Table 3. Two-way ANOVA on the effect of genotype, concentration and their interaction on the seedling characteristic of *Aegilops triuncialis* L

Source of Variation	*df*	*MS*	*Sign.*	η^2, %
RL				
Genotype	4	8.42	0.00	3.12
Concentration	6	161.56	0.00	**89.80**
Interaction	24	1.90	0.00	4.22
ShL				
Genotype	4	40.0	0.0	6.7
Concentration	6	341.0	0.0	**85.1**
Interaction	24	5.1	0.0	5.1
FRW				
Genotype	4	77.18	0.00	3.88
Concentration	6	1103.71	0.00	**83.21**
Interaction	24	24.82	0.00	7.48
FShW				
Genotype	4	538.19	0.00	3.68
Concentration	6	8268.46	0.00	**84.72**
Interaction	24	172.04	0.00	7.05
DRW				
Genotype	4	1.73	0.00	10.08
Concentration	6	9.29	0.00	**81.12**
Interaction	24	0.12	0.00	4.31
DShW				
Genotype	4	9.33	0.00	5.53
Concentration	6	84.40	0.00	**75.06**
Interaction	24	0.99	0.05	3.53

Root length (RL), Shoot length (ShL), Fresh root weight (FRW), Fresh shoot weight (FShW), Dry root weight (DRW), Dry shoot weight (DShW), η^2-degree of influence of the factors (%), df = degrees of freedom.

López-Méndez et al. (2024) noted that if the interaction between genotypes and salt is significant, it means that the genotypes respond differently at a given salt concentration. In our study, this is confirmed by the significant differences in the germination percentages of the five *Aegilops* genotypes studied under the different treatments (Figure 1). Increasing salt concentration resulted in a reduction in germination percentage, but the magnitude of the reduction under high salt stress was much greater than that under low salt stress. At the salinity level of 250 mM NaCl the germination percentage varied between 0% for BGR43669 and BGR43307 and 20% for BGR43665. At the highest concentration of 300 mM NaCl, only abnormal seed germination was recorded, manifested by a poorly developed root system without shoot development (Figure 1).

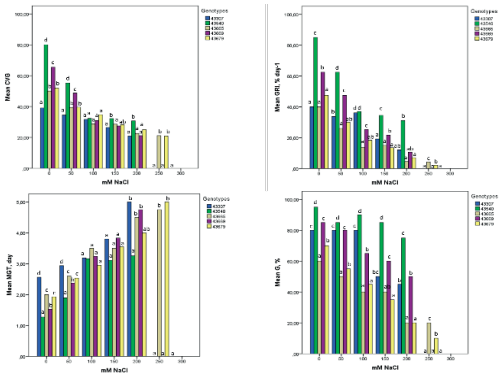


Figure 1. Variation of the germination characteristics (G, %-germination percentage; CVG-coefficient of velocity of germination; GRI, % day^{-1}-germination rate index; MGT, day-mean germination time) of five *Aegilops triuncialis* genotypes treated with different NaCl doses. Bars with different letters in the group are significantly different from each other according to Duncan's Multiple Range Test at $p < 0.05$

The application of increasing concentrations of sodium chloride solutions had a negative effect on CVG and GRI characteristics and positive on the MGT. This is due to the fact that increased salinity levels limit water absorption, which in turn leads to suppression of nutrient assimilation (Podder et al., 2020). Consequently, the germination rate decreases and mean germination time increase in response to increasing salinity. The results of this study are in agreement with the observations documented by Yohannes et al. (2020), Dehnavi et al. (2020), Kundu et al. (2023), Nikolić et al. (2023) and Ehtaiwesh (2024), who conducted their studies on different plant species. Dehnavi et al. (2020) and Kundu et al. (2023) noted that the observed variations among accessions in germination parameters might be attributable to both hereditary factors and specific genetic influences.

The relative injury rate (RIR), utilized in this study as a metric to evaluate the degree of stress experienced by plants under salinity conditions, exhibited significant disparities among genotypes at varying salinity levels. At low concentrations of 50-100 mM NaCl, the coefficient ranged from 0 for BGR43307 (for the two concentrations, respectively) to 0.33 for BGR43665 (at 100 mM NaCl, respectively). In the context of medium salt stress, the lowest RIR was observed for BGR43540, at both 150 and 200 mM NaCl concentrations. The highest RIR values, exceeding 0.80, were attained under conditions of 250-300 mM NaCl treatment, with the exception of BGR43665 at 250 mM NaCl, where an RIR of 0.67 was recorded (Table 4).

Table 4. Variation in the relative injury rate (RIR), root height reduction (RHR, mm) and shoot height reduction (ShHR, mm) of five barbed goatgrass genotypes treated with different NaCl doses

Genotype	RIR	RHR	ShHR
50 mM NaCl			
BGR43679	0.21c	0.09ab	0.29b
BGR43540	0.11abc	0.32c	0.21ab
BGR43669	0.06ab	0.05a	0.15a
BGR43307	0.00a	0.13b	0.26b
BGR43665	0.17bc	0.05a	0.13a
Average	0.11	0.13	0.21
100 mM NaCl			
BGR43679	0.36c	0.15a	0.32ab
BGR43540	0.05a	0.48b	0.52b
BGR43669	0.23b	0.22ab	0.29a
BGR43307	0.00a	0.17a	0.42ab
BGR43665	0.33bc	0.12a	0.33ab
Average	0.2	0.23	0.37
150 mM NaCl			
BGR43679	0.50c	0.52bc	0.70bc
BGR43540	0.11a	0.59c	0.71c
BGR43669	0.30b	0.36a	0.50a
BGR43307	0.38bc	0.41ab	0.59abc
BGR43665	0.33bc	0.33a	0.58ab
Average	0.32	0.44	0.62
200 mM NaCl			
BGR43679	0.71c	0.71b	0.92a
BGR43540	0.21a	0.73b	0.90a
BGR43669	0.41b	0.55a	0.89a
BGR43307	0.44b	0.74b	0.88a
BGR43665	0.67c	0.67b	0.86a
Average	0.49	0.68	0.89
250 mM NaCl			
BGR43679	0.86b	0.93b	0.98a
BGR43540	1.00c	0.89a	1.00a
BGR43669	1.00c	0.90a	1.00a
BGR43307	1.00c	0.92ab	1.00a
BGR43665	0.67a	0.91ab	0.99a
Average	0.9	0.91	0.99
300 mM NaCl			
BGR43679	1	0.96b	1
BGR43540	1	0.89a	1
BGR43669	1	0.89a	1
BGR43307	1	0.94b	1
BGR43665	1	0.94b	1
Average	1	0.92	1

Means within a column that have different superscript letters are significant different from each other according to Duncan's Multiple Range Test at $p < 0.05$.

In earlier studies made by Ali et al. (2019), Yohannes et al. (2020) and Habibi et al. (2021) also noted that RIR increase with increasing concentration of salinity level.

In the initial stages of salt stress, plants exhibit reduced water uptake by their roots and accelerated water loss in their leaves. This phenomenon can be attributed to the elevated salt content in the soil and plants, which exerts pressure and accelerates water loss. According to experts in the field, salt stress can be classified as a form of hyperosmotic stress (Seeda et al., 2022). Salinity has been demonstrated to impede the maintenance of adequate nutrient levels, which are indispensable for the growth of plants. This is attributable to the osmotic and specific ion toxicity effects of salinity. Consequently, salinity imposes limitations on root development and seedling growth (Pour-Aboughadareh et al., 2021; Irik & Bikmaz, 2024). As illustrated in Table 4, there was significant variation in the root and shoot height reductions (RHR, ShHR) of studied genotypes, when treated with different doses of NaCl. The lowest RHR and ShHR were exhibited by BGR 43665 and BGR 43669 at 50 mM NaCl. At the highest salinity stress, the root reduction varied between 0.89 for BGR43540 and BGR43669 to 0.96 for BGR 43679, while the shoot reduction was 1 for all studied genotypes. In general, the reduction in shoot length was greater than the reduction in root length. The similar results were recorded by Chakma et al. (2019); Podder et al. (2020); Habibi et al. (2021); Fatema et al. (2024) for different plant species.

Dry matter production is an important criterion to evaluate salt tolerance in plant since it permits direct estimations of economic returns under specific saline conditions. Tao et al. (2021) found that under salt stress at seedling stage, tolerant cultivars had higher shoot water content, shoot dry weight, and lower stomata density. Therefore, in this study, shoot dry weight data obtained under application of different NaCl salinity stresses were used to calculate 9 indices for screening salt tolerant and susceptible genotype (Pour-Aboughadareh et al., 2019).

In the control variant, shoot dry weight ranged from 4.71 mg/plant for BGR43307 to 7.75

mg/plant for BGR43665. Under salinity stress, shoot dry weight varied between 0 mg/plant for BGR43540, BGR43669 and BGR43307 in the variant with 250 mM NaCl and 6.48 mg/plant for BGR43665 in the variant with 50 mM NaCl. The relative change due to salinity stress demonstrated that the genotypes BGR43665 and BGR43679 exhibited minimal alterations at 50 mM NaCl, with respective reductions of 16.34% and 16.55% compared to the control. At 100 mM to 250 mM NaCl, genotype BGR43665 showed the least significant change compared to the control, in contrast to the other genotypes evaluated in this study (Table 5).

Table. 5 Variation in tolerance and susceptibility indices at different salinity level of five barbed goatgrass genotypes

Genotype	Yp	Ys	RC	TOL	MP	GMP	HM	SSI	STI	YI	YSI	RSI
50 mM NaCl												
BGR43679	5.79	4.83	16.55	0.96	5.31	5.29	5.27	0.62	0.72	**1.06**	**0.83**	**1.14**
BGR43540	6.96	4.05	41.85	2.91	5.51	5.31	5.12	1.56	0.72	0.89	0.58	0.79
BGR43669	6.00	4.25	29.17	1.75	5.13	5.05	4.98	1.09	0.65	0.93	0.71	0.97
BGR43307	4.71	3.25	30.97	1.46	3.98	3.91	3.85	1.16	0.39	0.71	0.69	0.94
BGR43665	7.75	6.48	16.34	1.27	7.12	7.09	7.06	0.61	1.29	**1.42**	**0.84**	**1.14**
100 mM NaCl												
BGR43679	5.79	3.62	37.58	2.18	4.70	4.58	4.45	0.94	0.54	**0.97**	**0.62**	**1.04**
BGR43540	6.96	3.23	53.69	3.74	5.09	4.74	4.41	1.34	0.58	0.86	0.46	0.77
BGR43669	6.00	3.25	45.83	2.75	4.63	4.42	4.22	1.14	0.50	0.87	0.54	0.91
BGR43307	4.71	3.01	36.09	1.70	3.86	3.76	3.67	0.90	0.36	0.81	0.64	1.07
BGR43665	7.75	5.58	27.96	2.17	6.67	6.58	6.49	0.70	1.11	**1.49**	**0.72**	**1.20**
Average	**6.24**	**3.74**	**40.23**	**2.51**	**4.99**	**4.81**	**4.65**	**1.00**	**0.62**	**1.00**	**0.60**	**1.00**
150 mM NaCl												
BGR43679	5.79	2.43	57.99	3.36	4.11	3.75	3.43	1.10	0.36	0.82	0.42	0.89
BGR43540	6.96	2.89	58.52	4.08	4.93	4.49	4.08	1.11	0.52	0.98	0.41	0.88
BGR43669	6.00	3.08	48.61	2.92	4.54	4.30	4.07	0.92	0.47	**1.04**	**0.51**	**1.08**
BGR43307	4.71	2.30	51.15	2.41	3.50	3.29	3.09	0.97	0.28	0.78	0.49	1.03
BGR43665	7.75	4.08	47.31	3.67	5.92	5.63	5.35	0.90	0.81	**1.38**	**0.53**	**1.11**
Average	**6.24**	**2.96**	**52.72**	**3.29**	**4.60**	**4.29**	**4.00**	**1.00**	**0.49**	**1.00**	**0.47**	**1.00**
200 mM NaCl												
BGR43679	5.79	1.00	82.73	4.79	3.40	2.41	1.71	1.04	0.15	0.78	0.17	0.84
BGR43540	6.96	0.79	88.72	6.18	3.88	2.34	1.41	1.12	0.14	0.61	0.11	0.55
BGR43669	6.00	1.25	79.17	4.75	3.63	2.74	2.07	1.00	0.19	**0.97**	**0.21**	**1.01**
BGR43307	4.71	0.71	84.96	4.00	2.71	1.83	1.23	1.07	0.09	0.55	0.15	0.73
BGR43665	7.75	2.70	65.16	5.05	5.23	4.57	4.00	0.82	0.54	**2.09**	**0.35**	**1.69**
Average	**6.24**	**1.29**	**80.15**	**4.95**	**3.77**	**2.78**	**2.08**	**1.01**	**0.22**	**1.00**	**0.20**	**0.96**
250 mM NaCl												
BGR43679	5.79	0.01	99.83	5.78	2.90	0.24	0.02	1.00	0.00	**0.50**	**0.00**	**0.54**
BGR43540	6.96	0.00	100.00	6.96	3.48	0.00	0.00	1.00	0.00	0.00	0.00	0.00
BGR43669	6.00	0.00	100.00	6.00	3.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
BGR43307	4.71	0.00	100.00	4.71	2.35	0.00	0.00	1.00	0.00	0.00	0.00	0.00
BGR43665	7.75	0.09	98.84	7.66	3.92	0.84	0.18	0.99	0.02	**4.50**	**0.01**	**3.62**
Average	**6.24**	**0.02**	**99.73**	**6.22**	**3.13**	**0.22**	**0.04**	**1.00**	**0.00**	**1.00**	**0.00**	**0.83**

Yp- shoot dry weight under control, mg/plant, Ys- shoot dry weight under salinity condition, RC-relative change TOL-Tolerance, MP-Mean productivity, GMP-Geometric mean productivity, HM-Harmonic mean, SSI-Stress susceptibility index, Stress tolerance index, YI-Yield index, YSI-Yield stability index, RSI- Relative stress index.

The TOL index states that genotypes with higher values are more sensitive to stress, while those with lower values are more tolerant (Pour-Aboughadareh et al., 2019). In accordance with these findings, genotype BGR43679 exhibited the greatest tolerance at 50 mM NaCl salinity, while genotype BGR43307 demonstrated the highest tolerance when subjected to increasing concentrations of sodium chloride ranging from 100 to 250 mM, as compared to the other genotypes. Pour-Aboughadareh et al. (2019) noted that genotypes demonstrating robust performance under both non-stress and stress conditions had high values for the STI, MP, GMP, and HM indices, thus being identified as tolerant. The results of the study showed that genotype BGR43665 demonstrated the greatest salt tolerance when exposed to increasing concentrations of sodium chloride ranging from 50 to 250 mM, as a comparison to the other genotypes. The SSI (Stress Susceptibility Index) is a tool employed to evaluate the severity of genotypes in stress environments, thereby reflecting their vulnerability. Lower SSI values are indicative of genotypes that are more tolerant to stress, and conversely, higher SSI values are indicative of genotypes that are less tolerant to stress (Bhandari et al., 2024). According to this index genotype BGR43540 was identified as the most sensitive genotype, while BGR43665 as the most tolerant to the tested salt doses. The three stability indices (YI, YSI and RSI) used to assess genotypic stability under both stress and non-stress conditions showed that genotypic stability of the studied genotypes decreased with increasing salt stress. According to these indices, the most tolerance genotypes at low salinity stress (50-100 mM NaCl) were BGR43679 and BGR43665, at medium salinity stress were BGR43669 and BGR43669, while for the high stress respect-tively BGR43679 and BGR43665 (Table 5).

Table 6 presents the ranking of the genotypes studied based on the indices calculated for each variant in the experiment. Ranking by different indices for each of the genotypes studied affects its rank differently relative to the whole group, so the average of the summary ranks (ASR) was calculated for all statistics to select potentially the best genotypes. The genotype with a low ASR value is considered as the most superior genotype (Pour-Aboughadareh et al. (2019). Therefore, taking into account the calculated values for ASR, the genotype most tolerant to the studied salinity doses was BGR43665, which exhibited values ranging from 1.09 at 50 mM NaCl to 1.36 at 250 mM NaCl. The most sensitive to NaCl salinity stress was BGR43307.

Table 6. Ranking of studied five barbed goatgrass genotypes on the base of the calculated tolerance and susceptibility indices at different salinity level

Genotype	Yp	Ys	TOL	MP	GMP	HM	SSI	STI	YI	YSI	RSI	SR	ASR	SD
50 mM NaCl														
BGR43679	4	2	1	3	3	2	2	3	2	2	2	26	2.36	0.81
BGR43540	2	4	5	2	2	3	5	2	4	5	5	39	3.55	1.37
BGR43669	3	3	4	4	4	4	3	4	3	3	3	38	3.45	0.52
BGR43307	5	5	3	5	5	5	4	5	5	4	4	50	4.55	0.69
BGR43665	1	1	2	1	1	1	1	1	1	1	1	12	**1.09**	0.3
100 mM NaCl														
BGR43679	4	2	2	3	3	2	3	3	2	3	3	30	2.73	0.65
BGR43540	2	4	5	2	2	3	5	2	4	5	5	39	3.55	1.37
BGR43669	3	3	4	4	4	4	4	4	3	4	4	41	3.73	0.47
BGR43307	5	5	1	5	5	5	2	5	5	2	2	42	3.82	1.66
BGR43665	1	1	2	1	1	1	1	1	1	1	1	12	**1.09**	0.3
150 mM NaCl														
BGR43679	4	4	3	4	4	4	4	4	4	4	4	43	3.91	0.3
BGR43540	2	3	5	2	2	2	5	2	3	5	5	36	3.27	1.42
BGR43669	3	2	2	3	3	3	2	3	2	2	2	27	2.45	0.52
BGR43307	5	5	1	5	5	5	3	5	5	3	3	45	4.09	1.38
BGR43665	1	1	4	1	1	1	1	1	1	1	1	14	**1.27**	0.9
200 mM NaCl														
BGR43679	4	3	3	4	3	3	3	3	3	3	3	35	3.18	0.4
BGR43540	2	4	5	2	4	4	5	4	4	5	5	44	4	1.1
BGR43669	3	2	2	3	2	2	2	2	2	2	2	24	2.18	0.4
BGR43307	5	5	1	5	5	5	4	5	5	4	4	48	4.36	1.21
BGR43665	1	1	4	1	1	1	1	1	1	1	1	14	**1.27**	0.9
250 mM NaCl														
BGR43679	4	2	2	4	2	2	2	2	2	2	2	26	2.36	0.81
BGR43540	2	3	4	2	3	3	3	3	3	3	3	32	2.91	0.54
BGR43669	3	3	3	3	3	3	3	3	3	3	3	33	3	0
BGR43307	5	3	1	5	3	3	3	3	3	3	3	35	3.18	1.08
BGR43665	1	1	5	1	1	1	1	1	1	1	1	15	**1.36**	1.21

Yp- shoot dry weight under control, mg/plant, Ys- shoot dry weight under salinity condition, TOL-Tolerance, MP-Mean productivity, GMP-Geometric mean productivity, HM-Harmonic mean, SSI-Stress susceptibility index, Stress tolerance index, YI-Yield index, YSI-Yield stability index, RSI- Relative stress index, SR-sum of ranks, ASR-average sum of ranks, SD-standard deviation.

CONCLUSIONS

Genotype, concentration and their interactions had significant individual effects on the investigated germination and seedling traits of *Aegilops triuncialis* L. The strongest effect on trait expression being exerted by the applied concentration, except for the trait germination percentage where genotype and concentration had almost equal contributions to trait expression. The application of increasing concentrations of the NaCl prolonged the mean germination time and suppressed the studied germination and seedling traits and had a greater inhibitory effect on shoot growth compared to root growth. The genotype BGR43665 was relatively the most tolerant to the application of increasing concentrations of 50 to 250 mM NaCl, The genotype BGR43665 was relatively the most tolerant to the application of increasing concentrations of 50 to 250 mM NaCl, while the highest concentration of 300 mM NaCl had toxic effect on seed germination in all of the studied genotypes.

ACKNOWLEDGEMENTS

This work was financially supported by the Bulgarian National Science Fund, Ministry of Education and Science under the project КП-06-ПН76/3 “Study of the genetic diversity of Aegilops species in the flora of Bulgaria”.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

YIELD STABILITY AND ITS ELEMENTS IN COMMON WINTER WHEAT VARIETIES UNDER PAZARDZHIK REGION CONDITIONS

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Abstract

In the period 2019-2023 in the field of irrigated agriculture experimental station Pazardzhik on cinnamon forest soil at humus 1.2-1.5% and pH 5.5-6 is set trial, which includes 9 varieties of common winter wheat selection of IPGR Sadovo. The stability of yields and its elements -1000 grains weight, and test weight, kg/hl Yield stability and grain physical properties of the varieties were evaluated using the stability variants σi2 and Si2 of Shukla (1972), the ecovalance Wi of Wricke (1962) and the phenotypic stability criterion (Ysi) of Kang (1993). IPCSSVKYSI (Interactive program for calculating Shukla`s stability index (Ysi)) developed by Kang and Magari (1995) was used to determine the stability index. The objective of our study was to evaluate the stability of common winter wheat cultivars grown in the Pazardzhik region. The results of this study will enable us to make an even more complete evaluation of the varieties of the IPGR Sadovo breeding of common winter wheat. Phenotypic stability is particularly important for practice.

Key words*: winter wheat, stability, yield, yield traits, climate change.*

INTRODUCTION

Wheat is one of the most important crops grown globally and plays a critical role in ensuring global food security (Gooding & Shewry, 2022; Gooding, 2023). Different forms of this species are cultivated, which are also related to its intended use and further uses. In the temperate climate of Central Europe, the most popular form is winter wheat, characterized by high yields and good baking properties (Oleksiak et al., 2022).

Despite continued progress in breeding new varieties, wheat yield remains highly dependent on the interaction between genotype and environment. Climate change, driven by global warming, exerts a substantial influence on agricultural production, particularly in areas reliant on rain-fed agriculture (Farrell et al., 2023). These areas are particularly susceptible to climatic conditions (Jiang et al., 2022). Climate warming directly alters the water cycle and precipitation patterns, resulting in increased dry-wet annual variability of precipitation (Malakouti, 2023; Zhang & Zhou, 2020). Therefore, gaining insight into the dynamics of meteorological factors and their impact on agricultural productivity is of importance for predicting future climatic trends and devising sustainable agricultural production strategies (Gu et al., 2017).

According to the World Bank of Development Report (WBDR), namely, The Changing Nature of Work published in early 2019, Bulgarian agriculture will be strongly influenced by climatic changes now and in the next 20-30 years. According to the ex-perts, agriculture plays a key yet disproportionate role in the socioeconomic fabric of rural Bulgaria (World bank, 2019).

In Bulgaria, there are regional differences in the likelihood of negative impacts from drought and flooding, as well as differences in the vulnerability, resilience and adaptive capacity of rural populations to climate change (Georgieva et al., 2022).

Genotype × environment (GE) interaction means that varieties respond differently to changes in growing conditions. Relative ranking of the performance of a group of cultivars under different environmental conditions gives

different results. Even among specific, regionally adapted breeding forms, it is difficult to select the best genotype because of the variability of yields in different years of observation and under different environmental conditions

In general, potential yield in dry years is strongly influenced by rainfall. Furthermore, inter-annual variations in rainfall are the main determinants of winter wheat yield in specific growing seasons without supplemental irrigation (Wang et al, 2025). Genotype × environment (GE) interaction means that varieties respond differently to changes in growing conditions. Relative ranking of the performance of a group of cultivars under different environmental conditions gives different results. Even among specific, regionally adapted breeding forms, it is difficult to select the best genotype because of the variability of yields in different years of observation and under different environmental conditions

In general, potential yield in dry years is strongly influenced by rainfall. Furthermore, inter-annual variations in rainfall are the main determinants of winter wheat yield in specific growing seasons without supplemental irrigation (Wang et al., 2025). Current winter wheat varieties will maintain their potential productivity, but actual yields will be strongly influenced by the duration of drought periods (Kazandjiev et al., 2023) GxE interactions have been used in various methods to characterize the response of genotypes to changing environments along with the average value of quantitative traits tested (Mohammadi et al., 2009; Christov et al., 2010; Hilmarsson et al., 2021). Research on this interaction is crucial for the stability and adaptability of varieties to different agro-climatic conditions. Genotype-environment interaction is defined as the variation in performance of different genotypes caused by specific environmental conditions. This phenomenon makes direct selection of high-yielding varieties difficult, as genotypes may show different behaviour depending on the location and year of cultivation. Various statistical and graphical methods are used to analyse the G×E interaction. Among the most common are: AMMI (Additive Main Effects and Multiplicative Interaction) analysis, a method that combines analysis of variance with principal components to identify stable genotypes (Gauch, 2020). GGE Biplot analysis, a graphical method that represents genotype and environment interaction effects, allowing identification of the most productive and stable varieties (Yan & Tinker, 2019).

The objective of our study was to evaluate the stability of common winter wheat cultivars grown in the Pazardzhik region. The results of this study will enable us to make an even more complete evaluation of the varieties of the IPGR Sadovo breeding of common winter wheat. Phenotypic stability is particularly important for practice.

MATERIALS AND METHODS

In the period 2019-2023 on the field of irrigated agriculture experimental station Pazardzhik on cinnamon forest soil at humus 1.2-1.5% and pH 5.5-6 is set trial, which includes 9 varieties of common winter wheat breeding of IPGR Sadovo. It is located in southern Bulgaria, in the central part of the Upper Thracian Lowland, north of the Rhodope Mountains, along the Maritsa River. It was laid out using the long strip method in four replications with a harvest plot size of 10 m^2. The sowing rate is 550 gs/m^2. Fertilization was with phosphorus (20 kg/da), applied as triple superphosphate presowing. The entire nitrogen fertilizer rate was applied as N_{12} (applied as ammonium nitrate). The stability of yields and its elements -1000 grains weight, g by weighing two samples of 500 grains (BSS ISO 520:2003) and test weight, kg/hl according to BSS ISO 7971:2000 of varieties of common winter wheat were determined.

Analysis of variance (ANOVA) and variational analysis was performed to determine the significance and influence of individual factors. The coefficient of variation was calculated. It is accepted that the variation is considered weak if the coefficient of variation is up to 10%, medium - when it is greater than 10% and less than 20%, strong - when it is over 20% (Dimova & Marinkov, 1999).

Yield stability and grain physical properties of the varieties were evaluated using the stability variants σi^2 and Si^2 of Shukla (1972), the ecovalance Wi of Wricke (1962) and the phenotypic stability criterion (Ysi) of Kang (1993). IPCSSVKYSI (Interactive program for calculating Shukla's stability index (Ysi))

developed by Kang & Magari (1995) was used to determine the stability index.

RESULTS AND DISCUSSIONS

Multilocation trials - used for breeding of varieties with high adaptability in different agro-ecological conditions (Bhatta et al., 2022). Different climatic conditions - temperature, rainfall, soil characteristics - have a significant impact on yield. Studies have shown that, high temperatures can reduce yield by up to 20% due to shortening of the growing season (Lobell et al., 2018). Moisture deficiency leads to reduced biomass and poor grain quality (Yang et al., 2020). High soil fertility promotes varietal resistance under stress conditions (Sharma et al., 2023). Modern breeding programs aim to develop varieties with higher yield stability under different environmental conditions. Genetic selection combined with molecular markers allows identification of genes associated with drought and temperature stress tolerance (Singh et al., 2019). In addition, integrating predictive models of G×E interactions helps develop cultivars that exhibit greater stability. Research on genotype-environment interactions in common wheat is essential for improving breeding programs and ensuring stable yields under changing climates. The development of new methodologies for analyzing G×E interactions, combined with advances in genetics, will play a key role in the future selection of high-yielding, resistant varieties.

Table 1 presents the tracking data for precipitation and monthly mean temperature for the period 2019-2023. Deviations are observed for both monitored indicators. For average monthly temperatures from October to February inclusive, there is a trend of: an increase compared to the norm of the 70-year period, and for the months of March to May a decrease from minus 0.7 to minus 4.1ºC. There is a shift of the 'winter or cool months. For the indicator rainfall sum, in two of the five years the values are lower than the norm (2018/2019; 2022/2023), with a difference of more than 135 mm in the growing season 2022/2023. In 2019/2020, rainfall is close to normal. In 2020/2021 and 2021/2022, more precipitation fell, but it was unevenly distributed. In all five years of the study, lower than normal rainfall during the phases heading, flowering and beginning of grain filling.

Table 1. Deviations in precipitation and average monthly temperatures for the period 2019-2023

Months/ Year	X	XI	XII	I	II	III	IV	V	VI
rainfall deviation, mm									
2018-2019	-28.3	20.1	-24.8	-16.5	-19.1	-34.4	14.6	-29.1	72.5
2019-2020	-31.7	56.2	-32	-34.1	-9.3	76	34.8	-32	-22.1
2020-2021	24.4	-38.2	1.7	44.6	-8.5	-2.3	30	-17.4	17.6
2021-2022	121	-35.5	20.6	5	17.1	-17	4.8	-48.6	-5.7
2022-2023	-35.6	-20.4	-17.8	-5.9	-28.1	-4.5	10.1	-19.6	-14.5
deviation at mean monthly temperature, ^{0}C									
2018-2019	0.9	0.3	0.2	2	2.1	3.6	-0.3	-1.6	1.8
2019-2020	1.8	3.3	1.9	2.8	4	2.1	-0.9	-1.8	0.3
2020-2021	2	-0.1	3.3	2.8	2.9	-0.7	0.1	-1.9	0.5
2021-2022	-1.7	0.9	1.5	2.5	2.5	-1.4	0	-0.8	1.7
2022-2023	1.3	2.7	3.4	5	3.2	1.7	-0.7	-4.1	0.8

The yield and quality based performance of all tested genotype in breeding experiments is the result of the interaction among genotypes, and environmental factors (Akcura & Kaya, 2008). Therefore, evaluating and increasing the yield potential of genotypes in different ecologies in variable climatic conditions is given high importance as an important selection criteria in Turkish wheat breeding programs (Bilgin et al., 2016). Besides these, many other factors like crop rotation, tillage, fertilization, genotype, biotic and abiotic factors could also affect yield and grain yield potential of wheat varieties in a region (Pepo & Kovacevic, 2011). Therefore would be new varieties are tested in different environments, to facilitate recommending them for different regions (Yaşar et al., 2020) depending on their compatiblity with local ecologies. Kaya & Akcura (2014) and Nehe et al. (2019) suggest that genotypes and environment including precipitation have the main effect on grain yield and quality characteristics of bread wheat are very important in multilocation experiments.

Table 2 presents the grain yield results for the wheat varieties studied. The data from our table show that the highest average yield for the study period was recorded in the variety Sashets - 652.2 kg/da, and the lowest result was shown by the variety Pobeda - 547.0 kg/da. In eight genotypes there was an excess of the trait compared to the standard, but the differences were not mathematically signifficant. Comparing the individual yield values in different years, it can be seen that in the 2022 crop year the lowest average yields were recorded - 438.7 kg/da, while the maximum grain yield was recorded in 2020, with three wheat varieties recording yields of over 700 kg/da.

Table 2. Average yield of winter common wheat for the period 2019-2023

№	Genotyp	Grain yield, kg/da						± D	Sign.	% to standard
		2019	2020	2021	2022	2023	$\bar{x}$			
1	Sadovo 1 – st.	606.7	655.9	576.0	442.0	631.0	582.3			100
2	Pobeda	587.9	583.4	539.0	414.0	610.5	547.0	-35.3	n.s.	93.9
3	Murgavets	632.3	698.8	630.0	407.0	645.5	602.7	20.4	n.s.	103.5
4	Boryana	659.6	704.4	714.0	469.0	603.0	630.0	47.7	n.s.	108.2
5	Geya	689.1	727.1	658.0	456.0	595.0	625.0	42.7	n.s.	107.3
6	Fermer	535.5	597.7	674.0	452.0	586.5	569.1	-13.2	n.s.	97.7
7	Gizda	662.7	769.5	713.0	447.5	570.0	632.5	50.2	n.s.	108.6
8	Nikibo	679.4	633.6	621.0	435.0	579.0	589.6	7.3	n.s.	101.2
9	Ginra	646.3	666.4	669.0	433.0	625.5	608.1	25.7	n.s.	104.4
10	Nadita	640.0	672.0	664.0	447.0	601.0	604.8	22.5	n.s.	103.9
11	Sashets	685.0	730.0	732.0	423.0	691.0	652.2	69.9	n.s.	112.0
Mean		638.6	676.3	653.6	438.7	612.5	603.9			
Minimum		535.5	583.4	539.0	407.0	570.0	547.0			
Maximum		689.1	769.5	732.0	469.0	691.0	652.2			
Std. Dev.							30.7			
Coef. Var.							9.3			
Standard error							5.1			
GD 5.0%=74.6; GD 1.0%=98.7; GD 0.1%=127.3										

+ -,+ + - -,+ + + - -, signifficant at GD 5.0%, GD 1.0% and GD 0.1%; n.s. - not significant

For the milling industry, the so-called milling qualities of wheat are of crucial importance. This concept covers a complex of physical particularityof the grain, such as test weight (density), large (size), vitreous, etc., which have a significant influence in the process of preparation and milling of the grain and determine the yield and quality of the flour (Tsenov et al., 2015; Nazarenko et al., 2020). Determination of the test weight is adopted by all state standards as an important trait of the quality of grain raw materials. A grain with a high test weight is well developed, plump and contains relatively more endosperm and less shell. Determination of grain density is an indicator of the overall quality and initial evaluation of the grain. (Bern & Brumm, 2009). The reported mean values of test weight (Table 3) ranged from 69.28 (Geya) to 75.60 (Pobeda) kg/hl. Three genotypes were above the standard level and their difference with Sadovo 1 was mathematically assured. In the 2020 crop year, the genotypes studied realized the highest average value of the trait - 78.01 kg/hl, while the lowest test weight was recorded in 2022 -71.50 kg/hl.

The 1000 kernel weight is an important indirect trait that characterizes the grain size, its milling quality (therelative content of endosperm, as well as the potential yield of flour with a low ash content), and determines its quality as seed material (Taneva et al., 2014; Ivanov, 2019). The hereditary validity of individual quality parameters is undoubtedly an established fact. The varietal determination of the grainweight is strongly manifested. It is believed that a larger and heavier grain is distinguished by a greater potential for flour yield, although this dependence has not been mathematically significant (Ivanov, 2019; Nazarenko et al., 2020).

The results obtained for the trait 1000 grains weight (Table 4) ranged from 33.20 (Gizda) to 44.24 g (Geya). On four wheat varieties, the reported value of the trait exceeded that of the standard, and in two of them the difference was mathematically significant. In 63.6% of the total varieties tested, the 1000 grains weight was above 40 g. The highest mean values were recorded in 2019 (44.98 g), while the most unfavorable year for the studied trait appeared to be 2022 with a mean value of the trait of 33.13 g.

Table 3. Test weight of common winter wheat varieties for the period 2019-2023

№	Genotyp	Test weight, kg/hl						± D	Sign.	% to standard
		2019	2020	2021	2022	2023	$\bar{x}$			
1	Sadovo 1 – st.	73.26	77.84	72.58	73.90	73.10	74.14			100
2	Pobeda	75.48	78.84	75.46	73.00	75.20	75.60	1.46	+++	102.0
3	Murgavets	72.78	78.12	73.12	70.80	72.50	73.46	-0.67	---	99.1
4	Boryana	71.01	80.40	74.07	72.80	72.20	74.10	-0.04	n.s.	99.9
5	Geya	68.66	73.35	68.19	67.10	69.10	69.28	-4.86	---	93.4
6	Fermer	72.94	77.88	72.60	72.40	73.00	73.76	-0.37	--	99.5
7	Gizda	74.65	78.89	73.56	72.00	74.80	74.78	0.64	+++	100.9
8	Nikibo	71.39	75.07	71.70	70.10	71.90	72.03	-2.10	---	97.2
9	Ginra	73.79	78.82	75.77	71.20	73.10	74.54	0.40	+++	100.5
10	Nadita	73.10	79.06	74.14	71.80	72.50	74.12	-0.02	n.s.	100.0
11	Sashets	73.20	79.80	73.86	71.40	72.70	74.19	0.06	n.s.	100.1
Mean		72.75	78.01	73.19	71.50	72.74	73.64			
Minimum		68.66	73.35	68.19	67.10	69.10	69.28			
Maximum		75.48	80.40	75.77	73.90	75.20	75.60			
Std. Dev.							1.69			
Coef. Var.							0.51			
Standard error							2.3			
GD 5.0%=0.22; GD 1.0%=0.30; GD 0.1%=0.38										

+ -,+ + - -,+ + + - -, signifficant at GD 5.0%, GD 1.0% and GD 0.1%; n.s. - not significant

Table 4. Weight per 1000 grains of common winter wheat varieties for the period 2019-2023

№	Genotyp	1000 grains weight, g						± D	Sign.	% to standard
		2019	2020	2021	2022	2023	$\bar{x}$			
1	Sadovo 1 – st.	48.42	43.30	38.20	36.40	43.20	41.90	0.00		100
2	Pobeda	43.30	38.30	39.20	32.28	43.30	39.28	-2.63	---	93.7
3	Murgavets	43.22	42.46	41.10	35.20	45.20	41.44	-0.47	n.s.	98.9
4	Boryana	46.56	44.38	42.14	34.62	42.40	42.02	0.12	n.s.	100.3
5	Geya	49.34	44.66	44.26	33.52	49.40	44.24	2.33	+++	105.6
6	Fermer	45.32	40.88	37.92	33.84	41.40	39.87	-2.03	+++	95.2
7	Gizda	35.84	34.94	33.10	26.32	35.80	33.20	-8.70	---	79.2
8	Nikibo	46.28	43.50	42.14	38.50	44.70	43.02	1.12	+	102.7
9	Ginra	44.72	43.06	41.06	31.80	42.80	40.69	-1.22	-	97.1
10	Nadita	45.51	36.52	36.92	30.16	40.30	37.88	-4.02	---	90.4
11	Sashets	46.30	42.20	45.16	31.84	45.40	42.18	0.28	n.s.	100.7
Mean		44.98	41.29	40.11	33.13	43.08	40.52			
Minimum		35.84	34.94	33.10	26.32	35.80	33.20			
Maximum		49.34	44.66	45.16	38.50	49.40	44.24			
Std. .Dev.							3.01			
Coef.Var.							0.91			
Standard error							7.4			
GD 5.0%=0.22; GD 1.0%=0.30; GD 0.1%=0.38										

+ -,+ + - -,+ + + - -, signifficant at GD 5.0%, GD 1.0% and GD 0.1%; n.s. - not significan

The calculated coefficient of variation for thestudied traits shows that the variation is estimated to be low (CV>10%) according Dimova & Marinkov (1999).

Analysis of genotypex interaction is particularly import ant for the breeding process (Yan & Hunt, 2001). Very often, high yield stability is associated with low levels of manifes tation, and vice versa (Tsenov et al., 2004; Atanasova et al., 2010).

Table 5 presents the analysis of variance, where the strength of influence of genotype, environment and their interaction factors on the occurrence of the studied indices were evaluated.

The data from the analysis determined a leading influence of environment conditions on all three studied indicators, with Ɒ values ranging from 60.4 (1000 grains weight) to 68.6% (grain yield). The second most influential factor was genotype for the test weight (61.2%) and 1000 grain weight (60.4%) traits, while the interaction of genotype x environment conditions factors had a minor influence on the occurrence of the grain yield trait, with a value of Ɒ=9.9%. The influence of the sources of variation genotype, environment and their interaction was signifficant at α=0.001, the only exception being observed in grain yield where the influence of year was mathematically assured at α=0.01 level of confidence.

Table 5. Analysis of variance (ANOVA) for grain yield from common winter wheat

Indicator	Sources of variation	SS	df	MS	F exp.	F tab.	Ŋ,%	Sig.
Grain yield	Genotype - factor A	141294.0	10	14129.4	6.6	3.3	8.1	***
	Environment - factor B	1197354.4	4	299338.6	140.9	5.0	68.6	***
	Interaction - AxB	172311.4	40	4307.8	2.0	1.8	9.9	**
	Error	233750.0	110	2125.0			13.4	
	Total	1744709.8	164				100.0	
Test weight	Genotype - factor A	428.4	10	42.8	2243.9	3.3	31.2	***
	Environment - factor B	840.1	4	210.0	11001.3	5.0	61.2	***
	Interaction - AxB	101.7	40	2.5	133.2	2.1	7.4	***
	Error	2.1	110	0.0			0.2	
	Total	1372.3	164				100.0	
1000 grains weight	Genotype - factor A	1355.6	10	135.6	418.4	3.3	30.3	***
	Environment - factor B	2699.0	4	674.7	2082.5	5.0	60.4	***
	Interaction - AxB	378.7	40	9.5	29.2	2.1	8.5	***
	Error	35.6	110	0.3			0.8	
	Total	4469.0	164				100.0	

SS - sum of squares; gf - degrees of freedom; MS - variance; F exp. - F experimental; F tab. - F tabular; ŋ - force of influence of the factor (%);** - significant at $\alpha = 0.01$ *** - significant at $\alpha = 0.001$

A very important condition for determining the stability and adaptability of genotypes with respect to the trait yield is the presence of a proven interaction between the studied genotypes and the conditions of the environment in which they are grown (Uhr, 2015; Ivanov et al., 2018). Based on the signifficant influence of growing conditions on the manifestation of the studied traits in the analysis of variance gives us the basis to evaluate the studied genotypes also by their stability of the studied traits under different climatic conditions. The calculated variance stability σi^2 and Si^2 by Shukla, the Wricke's ecovalence W_i^2 and the Kang's stability criterion YSi are presented in Tables 6, 7 and 8. Varieties showing lower values for the parameters σi^2, Si^2, Wi are judged to be more stable because they interact less with the environmental conditions.

Negative values of the indicators σi^2 and Si^2 are assumed to be 0. At high values of the parameters - σi^2, Si^2, W_i^2 genotypes are considered as unstable. The results obtained for the stability of yield in the criteria σi^2, Si^2, W_i^2 (Table 6) show that the highest stability in the indicator of grain yield possess the varieties Nadita and Ginra. These genotypes interact less with environmental conditions and their yield is at a relatively constant level. The varieties Gizda and .Fermer are characterised as not stable in terms of yield, with large differences from year to year in the values obtained for the trait.

A comprehensive assessment of the integrity of genotypes is given by the Kang criterion (YSi). It is based on a simultaneous evaluation of the values of realized grain yield and its stability over the different climatic years. According to this criterion, genotypes are ranked in descending order, with those with the lowest values being the most economically valuable. In our study, Boryana, Ginra and Nadita can be likened as such varieties combining high and stable yield over the years.

Table 6. Stability parameters for the grain yield of common winter wheat in terms of years

№	Variety	GY	σ^2_i	s^2_i	W_i^2	YSi
1	Sadovo 1 st.	582.3	1512.7	598.5	5472.8	16
2	Pobeda	547.0	2502.3	842.4	8711.8	20
3	Murgavets	602.7	814.8	325.1	3188.8	11
4	Boryana	630.0	602.0	354.3	2492.5	6
5	Geya	625.0	921.3	478.1	3537.6	9
6	Fermer	569.1	3511.1	1268.3	12013.1	21
7	Gizda	632.5	3078.9	1215.7	10598.6	12
8	Nikibo	589.6	1264.4	631.0	4660.3	14
9	Ginra	608.0	-4.1	65.3	508.7	7
10	Nadita	604.8	-59.9	40.8	326.3	7
11	Sashets	652.2	1655.4	218.0	5940.0	9

GY- grain yield (da); σ^2_i - Shukla's stability variance; s^2_i - deviation from redression; W_i^2 - Wricke's ecovalence; YSi - Kang's criterion

The calculated parameters of phenotypic stability in test weight (Table 7) show that the varieties Murgavets and Geya1 are characterized by the highest stability of the

indicator according to the criteria σ^2_i and W_i^2, and according to the stability variant s^2_i variety Sashets ranks first, followed by Murgavets. As unstable and high fluctuations in test weight values we can liken Boryana, Sadovo 1 and Ginra. The varieties distinguished by high test weight values and good stability according to Kang's criterion (YSi) are the genotypes Pobeda, Nadita and Sashets.

Table 7. Stability parameters for the test weight of common winter wheat in terms of years

№	Variety	TW, $\bar{x}$	σ^2_i	s^2_i	W_i^2	YSi
1	Sadovo 1 st,	74.14	1.524	0.530	5.30	15
2	Pobeda	75.60	0.646	0.194	2.42	7
3	Murgavets	73.46	0.033	0.032	0.42	10
4	Boryana	74.10	3.102	1.008	10.46	18
5	Geya	69.28	0.239	0.137	1.09	13
6	Fermer	73.76	0.270	0.132	1.19	12
7	Gizda	74.78	0.667	0.351	2.49	10
8	Nikibo	72.03	0.663	0.062	2.48	17
9	Ginra	74.54	1.308	0.638	4.59	12
10	Nadita	74.12	0.247	0.093	1.12	9
11	Sashets	74.19	0.620	0.028	2.34	9

TW- test weight (kg/hl); σ^2_i - Shukla's stability variance; s^2_i - deviation from redression; W_i^2 - Wricke's ecovalence; YSi - Kang's criterion

The results obtained for 1000-grain weight (Table 8) show that high stability over the study period was found in the cultivars Gizda, Pobeda, and Nikibo, while Sashets and Sadovo 1, characterized by the highest values of the stability variants σ^2_i, s^2_i, and W_i^2, can be indicated as not stable. Boryana and Nikibo appear to be the most valuable according to Kang's criterion (YSi), combining high trait values and good stability across climatic years.

Table 8. Stability parameters for the 1000-grain weight of common winter wheat in terms of years

№	Variety	1000 GW, $\bar{x}$	σ^2_i	s^2_i	W_i^2	YSi
1	Sadovo 1 st.	41.90	5.942	2.876	20.59	15
2	Pobeda	39.28	1.375	0.795	5.65	11
3	Murgavets	41.44	2.708	0.914	10.01	12
4	Boryana	42.02	1.963	1.052	7.57	9
5	Geya	44.24	5.328	0.674	18.58	10
6	Fermer	39.87	1.575	0.804	6.30	12
7	Gizda	33.20	1.060	0.442	4.62	12
8	Nikibo	43.02	2.931	0.083	10.74	9
9	Ginra	40.69	1.428	0.692	5.82	10
10	Nadita	37.88	4.234	1.760	15.01	18
11	Sashets	42.18	6.171	2.350	21.34	14

1000 GW- 1000 grain weight (g); σ^2_i - Shukla's stability variance; s^2_i - deviation from redression; W_i^2 - Wricke's ecovalence; YSi - Kang's criterion

CONCLUSIONS

In the conditions of the Pazardzhik region:
- The highest average grain yield was realized by the Sashets variety;
- The maximum value of the trait test weight was recorded in variety Pobeda and the best result in the 1000 grains weight showed variety Geya;
- The leading influence on the phenotypic manifestation of the studied traits had the growing conditions.

The following genotypes can be singled out as the most valuable from the breeding point of view, distinguished by a good combination of high and stable results under different climatic conditions:
- Ginra: high and stable value of the trait grain yield;
- Sashets: high and stable value of the trait test weight;
- Nikibo: high and stable value of the trait 1000 grains weight.

The obtained data can be used in the selection and improvement work in the creation of new varieties of winter wheat, distinguished by quality stability and high values of the traits yield, test weight and 1000 grains weight.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

GENETIC NATURE OF QUANTITATIVE TRAITS IN DURUM WHEAT

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Abstract

The aim of the study is to determine the genetic nature of quantitative traits associated with productivity in durum wheat and to make recommendations for optimizing the breeding process in the early segregating generations. A diallel cross without reciprocals, was carried out at the Field Crops Institute - Chirpan, with five modern durum wheat varieties. Six quantitative traits were studied. The inheritance of productivity tillering is governed by overdominance, and is possible selection of genotype by phenotype, but in the later segregating generations. For traits such as plant height and thousand kernel weight, inheritance is controlled by incomplete dominance, and genotype selection by phenotype is possible in F_2. The inheritance of spike length is determined by overdominance, and is possible selection of genotype by phenotype in the later segregating generations. The spikelets number per spike is inherited through overdominance, and selection of genotype by phenotype is possible but should be carried out in the later segregating generations. The grains weight per spike is influenced by overdominance, and genotype selection by phenotype is possible after F_5.

Key words: *additive variance, dominance, narrow sense heritability, broad sense heritability, durum wheat.*

ITRODUCTION

Durum wheat is a primary raw material for the production of various semolina-based products. It possesses excellent physical and technological qualities, making it ideal for producing high-quality pasta products. These pasta products have high nutritional value and are also incorporated into various healthy human diets. Breeding and improvement work on durum wheat and the development of new varieties require extensive knowledge in multiple disciplines related to crop science. Understanding the genetic nature and the factors influencing the inheritance of grain yield-related traits is crucial for the effectiveness of the breeding process. The use of diallel crosses by breeders is a systematic method for obtaining information about gene action and genetic nature. Knowledge of the nature and extent of gene action in the expression of quantitative traits is a key component in successfully developing an appropriate strategy for effective breeding programs. Diallel analysis offers an advantage in studying quantitative traits. Although it requires relatively more work, it provides a highly effective assessment. Detailed information can be obtained from diallel crosses even in a single F_1 generation. Increasing our understanding of the genetic nature of quantitative traits allows for the optimization of the breeding process in durum wheat. This creates an opportunity for properly constructing the breeding strategy and accelerating the breeding process. The variation in gene action largely depends on the parental forms included in the study, the environment, and their interactions. To ensure the success of selection, it is necessary to deepen knowledge about the genetic nature of the most important quantitative traits related to productivity. The relevance of this issue is highlighted by the fact that in recent years, a wide range of new durum wheat varieties has been developed.

Numerous researchers have studied the mechanisms of grain yield inheritance and its components, elucidating the genetic nature of productivity and identifying possible types of gene action responsible for the expression of yield component traits. This indicates that identifying such patterns is extremely

important for determining the strategy in a breeding program. Dagustu (2008) reports that additive variance plays a primary role in the inheritance of the thousand kernel weight trait. On the other hand, Akbar et al. (2009) indicate that dominant parameters have a greater role in the inheritance of this trait. Gami et al. (2010) observe a predominant influence of overdominance on traits such as plant height, thousand kernel weight, productivity tillering and spikelets number per spike. They suggest the action of at least one group of genes showing dominance, responsible for the manifestation of yield components. Ahmad et al. (2011) examined traits including productivity tillering and thousand kernel weight. They found confirmed additive variance and dominant parameters for these traits under early and normal sowing conditions, while only additive variance was significant under late sowing. Vanda and Houshmand (2011) demonstrate a predominance of non-additive (dominant) over additive gene effects for the traits included in their study. Farooq et al. (2011) tracked traits such as spike length, spikelets number per spike, and the number of grains per spike. Additive variance was significant, showing a greater role of additive gene effects for spike length. However, higher dominant parameters were found for the spikelets number per spike, indicating the predominance of non-additive gene effects. Nazeer et al. (2011) reveal the genetic nature of traits including spike length, spikelets number per spike, grains number per spike and grains weight per spike. Additive variance was demonstrated for all traits except grains weight per spike, with higher dominant parameters noted for spike length. Singh and Sharma (2012) conducted experiments to study the genetic components of grain yield-related traits, confirming both additive variance and dominant parameters. Kutlu & Olgun (2015) performed diallel analysis without reciprocal crosses for traits such as plant height, spike length, spikelets number per spike, and grains weight per spike. They found significant values for both additive variance and dominant parameters, suggesting both types are responsible for inheritance. Sadeghzadeh-Ahari et al. (2015) studied a diallel scheme with 6x6 parents of durum wheat, finding that additive variance played a greater role for spike length and productivity tillering traits. Fellahi et al. (2015) explored inheritance mechanisms using diallel analysis for productivity elements, analyzing traits like thousand kernel weight and spikelets number per spike in F_1 and F_2 generations. They reported that under their study conditions, the inheritance of traits involved 2 to 5 genes showing dominance. Ahmad et al. (2017) suggested that traits like plant height, productivity tillering, spike length and spikelets number per spike are primarily governed by non-additive gene effects (dominant parameters). Heritability coefficients in durum wheat are crucial for determining proper selection. High broad-sense coefficients indicate the possibility of genotype selection based on phenotype, while high narrow-sense heritability suggests that selection can begin in earlier segregating generations. Amin (2013) examined the genetic behavior of certain agronomic traits in durum wheat crosses under normal and heat stress conditions, finding moderate broad and narrow sense heritability coefficients for productivity tillering and thousand kernel weight. In a diallel cross with durum wheat, Hannachi et al. (2013) identified high narrow sense heritability for traits like plant height and thousand kernel weight, a moderate coefficient for the grains number per spike, and a low one for productivity tillering. For broad sense heritability in durum wheat, Basciftci et al. (2013) reported high values for traits including spike length, spikelets number per spike, grains number per spike, grains weight per spike and thousand kernel weight. Hannachi et al. (2017) found high broad and narrow sense heritability coefficients for plant height and moderate coefficients for productivity tillering, grains number per spike and thousand kernel weight. From the reviewed literature, it can be concluded that different assessments of the genetic nature of productivity-related traits in durum wheat are observed depending on the genetic material used and the diverse experimental conditions. Therefore, conducting such an in-depth study under local conditions with modern durum wheat varieties is highly necessary.

The aim of this study is to establish the genetic nature of quantitative traits related to productivity and optimize the breeding process

in durum wheat. To create an opportunity for the correct construction of the breeding strategy and acceleration of the breeding process.

MATERIALS AND METHODS

In a diallel cross, five modern durum wheat varieties were used as parents. The varieties Victoria, Predel, Deni, and Progress were selected from the durum wheat breeding program of the Field Crops Institute - Chirpan, along with the foreign variety Superdur. The Victoria and Predel varieties were developed through combining breeding methods, while Deni and Progress were obtained through experimental mutagenesis. The Superdur variety originates from Austria. These varieties are genetically and phenotypically diverse, meeting the requirements for the diallel crossing scheme. The diallel cross was performed by intercrossing the five described varieties without reciprocals. The crossing was carried out under field conditions at the begining of heading time, with handmade emasculation and pollination. Each year, 30 plants per combination were handmade emasculated and pollinated.

The experiment was set up using a randomized block design with three replications under field conditions. The parents and F_1 hybrids were sown handmade in two rows, while the F_2 hybrids were sown in five row. The length of row was two meters, with a row spacing of 20 cm and 5 cm within the row. At full maturity, twenty plants from each parent, F_1, and F_2 generations were randomly selected. The experiment was conducted at the breeding experimental field of the Field Crops Institute - Chirpan over three consecutive years (2014-2016). The standard agronomic practices for durum wheat cultivation in the region were applied. The soil type is Eutric Vertisols (according to FAO classification). The preceding crop was green pea. Nitrogen fertilization was performed once in February with a rate of 10 kg/da active substance nitrogen. Weed control included herbicide application with a combination of two herbicides. No pesticides were used to control diseases and pests. During the three years of the study, temperatures were higher than the long-term average. The first and second years had normal precipitation during the growing season, while the third year had 20% less rainfall compared to the long-term average. The following traits were studied: Plant height (cm): Measured from the soil surface to the top of the spike (excluding awns) on the main stem. Productivity tillering (number): Counted the fertile spikes per plant. Spike length (cm): Measured on the main stem from the bottom spike to the top of the uppermost spikelet. Spikelets number per spike (number): Counted the spikelets in the main spike. Grains weight per spike (g): Weighed all grains from the main stem spike after handmade threshing. Thousand kernel weight (g): Weighed the mass of 1000 grains.

Statistical analysis was performed according to Hayman (1954; 1957), Jinks (1954) & Mather (1967) using the statistical software developed by Aksel & Johnson (1962). Heritability coefficients were calculated following the formulas of Mather & Jinks (1982). The parameters and indicators are denoted as follows: D - Component of genetic variance due to additive effect of genes (additive variance), H_1 - Components of genetic variance due to dominance effect, H_2 - Proportion of dominance variance due to the positive and negative effects of the genes in the parents (dominance parameters), h2 - Dominance effects, as algebric sum over all the loci in heterozygous phase in all the crosses, F - Covariance of additive and non-additive effects in a single array. It may be positive or negative, $(H_1/D)^{1/2}$-Mean degree of dominance, $H_2/4H_1$ - Proportion of genes with positive and negative effects in the parents, Kd/Kr - Proportion of dominant and recessive genes in the parents, h_2/H_2 - number of groups of genes with positive and negative effects in the parents.

RESULTS AND DISCUSSIONS

An analysis of the variance (not shown) was conducted, revealing significant differences between the tested genotypes for all traits included in the study. These differences were observed in both F_1 and F_2 generations. The significant differences between genotypes provide a basis for conducting a parametric genetic analysis and determining the parameters and indicators related to the genetic nature and expression of the studied traits. Table 1 presents the genetic parameters and

indicators according to Hayman (1954) & Jinks (1954) from the diallel cross for F_1 and F_2.

The additive variance (D), attributed to the additive action of genes, is significant for productivity tillering and grains weight per spike in F_2 and significant in both F_1 and F_2 generations for all other traits. It is nonsignificant for productivity tillering and grains weight in F_1. This indicates the substantial role of the additive gene action in controlling most productivity-related traits. In the F_2 generation, the traits productivity tillering and grains weight per spike show significant additive variance (D), suggesting that additivity in these traits increases in subsequent generations. Numerous authors report significant additive variance (Zanke et al., 2014; Shehzad et al., 2015; Sadeghzadeh-Ahari et al., 2015; Kutlu & Olgun, 2015).

Table 1 also includes the dominant parameters (H1) and (H2), reflecting the variation due to the dominant gene action. The dominant parameters are significant for all traits in both generations. Our results align with those obtained by Nazeer et al., 2011; Ahmad et al., 2011; Singh & Sharma, 2012. This indicates that dominance plays a major role in determining all traits included in this study. The dominance parameters are higher than the additive variance (D) in both generations for traits such as productivity tillering, spikelets number per spike, and grains weight per spike, indicating the greater importance of dominance in the expression of these traits. Authors such as Vanda & Houshmand (2011) also report higher dominance parameters than additive variance for these traits. For the remaining traits, the additive variance is greater than the dominance parameters, suggesting a greater role of additivity in trait expression. This corresponds with results obtained by Kutlu & Olgun (2015), Sadeghzadeh-Ahari et al. (2015). For all traits and in both generations, (H1) is greater than (H2). This suggests that the positive and negative alleles at loci showing dominance in the parents are not in equal proportions. The parametric component analysis demonstrates the significance of both additive variance and dominant parameters. This implies that both dominance and additivity are crucial for the inheritance of the studied traits. Breeding programs should include a system that considers both additive variance and dominance parameters to enhance durum wheat productivity.

The parameter (F) is related to the direction of dominance (Table 1). It indicates whether there are more dominant or recessive genes. For traits such as productivity tillering in F_2, plant height, and thousand kernel weight in both generations, and spike length in F_1, (F) is significant and positive.

Table 1. Genetic parameters for 6 quantitative traits from diallel crosses

Traits		D	F	H1	H2	h2
Productivity tillering	F_1	0.041 ± 0.12	-0.18 ± 0.31	2.46 ± 0.33	1.94 ± 0.30	1.61 ± 0.20
	F_2	0.129 ± 0.121	0.304 ± 0.303	1.21 ± 0.32	0.81 ± 0.29	0.50 ± 0.20
Plant height	F_1	87.14 ± 3.04	17.64 ± 7.59	39.16 ± 8.21	25.79 ± 7.44	3.41 ± 5.09
	F_2	85.09 ± 2.51	28.85 ± 6.29	57.73 ± 6.80	43.57 ± 6.16	70.47 ± 4.16
Spike length	F_1	0.87 ± 0.0067	0.91 ± 0.016	0.31 ± 0.018	0.29 ± 0.016	0.71 ± 0.011
	F_2	0.80 ± 0.0078	-0.07 ± 0.019	0.71 ± 0.021	0.068 ± 0.01	0.004 ± 0.01
Spikelets number per spike	F_1	0.30 ± 0.094	-0.089 ± 0.23	1.56 ± 0.25	0.98 ± 0.23	0.35 ± 0.15
	F_2	0.35 ± 0.08	0.079 ± 0.20	0.74 ± 0.22	0.46 ± 0.20	0.023 ± 0.13
Grains weight per spike	F_1	0.083 ± 0.086	0.020 ± 0.021	0.19 ± 0.023	0.18 ± 0.02	0.57 ± 0.014
	F_2	0.034 ± 0.013	0.025 ± 0.028	0.12 ± 0.03	0.10 ± 0.02	0.18 ± 0.018
Thousand kernel weight	F_1	41.96 ± 1.48	28.76 ± 3.70	6.96 ± 4.00	3.66 ± 3.63	-1.11 ± 2.45
	F_2	23.21 ± 1.61	4.05 ± 4.02	16.53 ± 4.35	13.98 ± 3.94	22.2 ± 2.66

* - P ≤ 0,05; D - Component of genetic variance due to additive effect of genes, H1 - Components of genetic variance due to dominance effect, H2 - Proportion of dominance variance due to the positive and negative effects of the genes in the parents, h2 - Dominance effects, as the algebraic sum over all the loci in the heterozygous phase in all the crosses, F - Covariance of additive and non-additive effects in a single array (can be positive or negative).

This indicates that the positive and negative genes are unevenly distributed. Other traits in the study show variability in significance and changes in direction, likely due to environmental conditions. The overall measure of dominance is determined by the indicator $(H1/D)^{0.5}$. The degree of dominance $(H1/D)^{0.5}$ (Table 2) reveals that overdominance plays a

primary role in the inheritance of traits such as productivity tillering, spikelets number per spike and grains weight per spike. For traits like plant height and thousand kernel weight, incomplete dominance predominantly influences inheritance. The spike length trait shows incomplete dominance in F_1 but overdominance in F_2. Our findings are consistent with those of other authors (Menon & Sharma, 1997; Gami et al., 2010; Sadeghzadeh-Ahari et al., 2015).

The parameter F is confirmed by the values of the indicator H2/4H1, which provides an assessment of the average purity of positive and negative alleles at loci showing dominance (Table 2). The values of the indicator (H2/4H1) are close to 0.25 for traits such as spike length in F_1, grains weight per spike in both generations, and thousand kernel weight in F_2, confirming the presence of symmetry between dominant and recessive alleles. For the remaining traits, there is asymmetry between dominant and recessive alleles.

The ratio of dominant to recessive genes determining the trait (Kd/Kr) (Table 2) indicates varying dominance across generations. For most traits and generations, dominant genes predominate. For productivity tillering and spikelets number per spike in F_1 and spike length in F_2, values are below one, with recessive genes prevailing. For the other traits, dominant genes are predominant. The indicator (h2/H2) suggests the involvement of two major genes determining productivity tillering, one to three genes determining plant height, one - two genes for spike length, two to three genes for spikelets number per spike, one gene for grains weight per spike, and one to two genes for thousand kernel weight. These genes are often grouped according to the polygenic nature of the studied traits. The control of the traits mainly involves one to three major genes or gene groups showing dominance under environmental conditions.

Table 2. Genetic indicators for 6 quantitative traits from diallel crosses

Traits		$(H1/D)^{1/2}$	H2/4H1	Kd/Kr	h2/H2
Productivity tillering	F_1	7.74	0.19	0.55	1.20
	F_2	3.06	0.16	2.24	1.62
Plant height	F_1	0.66	0.16	1.35	2.56
	F_2	0.81	0.18	1.51	0.61
Spike length	F_1	0.59	0.23	1.19	0.40
	F_2	2.73	0.002	0.72	1.7
Spikelets number per spike	F_1	2.28	0.15	0.87	2.8
	F_2	1.45	0.15	1.16	2.0
Grains weight per spike	F_1	1.5	0.23	1.66	0.31
	F_2	1.87	0.20	1.47	0.55
Thousand kernel weight	F_1	0.4	0.09	11.6	2.39
	F_2	0.84	0.21	1.23	0.62

* - $P \leq 0{,}05$; $(H1/D)^{1/2}$ -Mean degree of dominance, H2/4H1-Proportion of genes with positive and negative effects in the parents, Kd/Kr-Proportion of dominant and recessive genes in the parents, h2/H2 -number of groups of genes with positive and negative effects in the parents, H2 - broad sense heritability coefficient and h2 - narrow sense heritability coefficient.

Heritability coefficients are directly related to selection effects and breeding efficiency. The broad sense heritability coefficients H2 are high for all traits included in this study in both generations (Table 3). This suggests that genotype selection by phenotype is feasible. The narrow-sense heritability coefficients provide the most accurate estimate of additive (breeding-useful) variance. For traits like plant height and thousand kernel weight, effective selection is possible in early segregating generations, while for all other traits, selection should start in later segregating generations (F_5-F_6). The results for heritability coefficients match those obtained by a number of authors (Gami et al., 2010; Yao et al., 2011; Khodadadi et al., 2012; Fellahi et al., 2015; Hannachi et al., 2017).

Table 3. Broad and narrow sense heritability coeficients in F_1 and F_2 for 6 quantitative traits from diallel crosses

Traits	Heritability coeficients F_1		Heritability coeficients F_2	
	H2 (broad sense)	h2 (narrow sense)	H2 (broad sense)	h2 (narrow sense)
Productivity tillering	0.95	0.41	0.92	0.32
Plant height	0.97	0.84	0.97	0.74
Spike length	0.76	0.12	0.99	0.98
Spikelets number per spike	0.86	0.57	0.80	0.56
Grains weight per spike	0.89	0.39	0.90	0.33
Thousand kernel weight	0.66	0.61	0.94	0.71

The obtained results indicate that the traits are suitable for individual selection by classical breeding methods. Therefore, in most cases, breeders can count on faster and higher results in improving these traits, with the exception of grain weight per spike and productivity tillering. The results of the diallel analysis presented in this way clarify the possibilities for increasing grain productivity by improving the individual traits related to it. Hannachi et al. (2017) reach such a conclusion. The unification and differentiation of traits with high heritability and genetic independence is most often associated with increasing productivity. Selection by a single trait can increase yield, but a more significant increase would be obtained by simultaneously improving its elements.

CONCLUSIONS

Additive variance and dominant parameters are important for all studied traits. A system incorporating both variances is needed to achieve maximum results. Overdominance plays a key role in the inheritance of traits such as productivity tillering, spike length, spikelets number per spike and grains weight per spike. For traits like plant height and thousand kernel weight, incomplete dominance is more significant. Broad sense heritability coefficients are high for all traits, enabling genotype selection by phenotype. Effective selection for plant height and thousand kernel weight is possible in early generations, while for other traits, selection should begin in later segregating generations (F_5-F_6). Individual selection is recommended for improving all studied traits.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

EVALUATION OF NEW VIRGINIA TOBACCO GENOTYPES FOR YIELD, MORPHOLOGICAL AND CHEMICAL TRAITS

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Abstract

To evaluate the yield, morphological traits and chemical indicators of Virginia tobacco genotypes, under agroecological conditions of Plovdiv, a study was conducted during 2017-2019 in an experimental field at Tobacco and Tobacco Products Institute, Markovo. Three new genotypes of Virginia tobacco - L 36, H 126 F_1, H 135 F_1 and standard Virginia 0514 were planted in four replications and evaluated by the following traits: plant height; number of leaves; twelfth leaf length, twelfth leaf width; leaf area, cured leaf yield, chemical components - nicotine, sugars, total nitrogen, balance ratio total nitrogen/nicotine and sugars/nicotine. It was found that statistically significant differences existed among the studied Virginia tobacco genotypes only in the indicators cured leaf yield, twelfth leaf length and in the adaptability of plant height trait. Genotypes H 126 and L 36 are distinguished by a proven greater twelfth leave length (64; 62 cm) and cured leaf yield (3.20; 3.14 t/ha). The presence of statistically significant differences between genotypes in terms of plant height trait proved H 126 to be the most resistant to environmental factors, followed by L 36. The standard V 0514 demonstrated superiority for the sugars/nicotine ratio, but for all genotypes the value was within the reference range. In L 36 the total nitrogen/nicotine ratio was below 1.

Key words*: Virginia tobacco, morphological traits, yield, ecological stability.*

INTRODUCTION

Nicotiana tabacum L. tobacco is cultivated worldwide (Kishore, 2014). The plant is cultivated as a source of leaves, primarily for use in the manufacture of nicotine-containing products such as cigarettes, cigars and smokeless tobacco products (Lewis, 2020). It can generate a large amount of biomass (Andrianov et al., 2010; Srbinoska, 2017), serves as a model system for tissue culture and genetic engineering (Ganapathi, 2004), as an energy crop for biofuel production (Barla & Kumar, 2019), is considered as a promising plant material with strong antioxidant potential and for the production of purified natural extracts with potential application in biopharmaceutics (Docheva et al., 2024).

Despite the decline in production, tobacco is still an important crop for Bulgaria's economy (Drumeva-Yoncheva, 2020). The country produces tobacco from the variety groups - Basma and Kaba Kulak - oriental tobaccos and Virginia and Burley, which are large-leaf tobaccos (Bozukov, 2014). In 2023, Virginia tobacco accounts for approximately 19% of total tobacco production in Bulgaria (https://www.mzh.government.bg/). Currently, Bulgarian selected varieties - Virginia 0514 and Hibrid (Virginia) 0454 - are used in the country. From 2023, the Official Variety List includes new tobacco variety Virginia 0842 (https://iasas.government.bg).

The main goal of any breeding program is to create varieties combining high productive potential (Dražic, 2012; Risteski & Kocoska. 2012; Dyulgerski & Docheva, 2017) and other basic agronomic characteristics (Becker & Leon, 1988), which is the result of the impact of genotype and environment (Ayalneh et al., 2013).

The geographical environment can significantly impact the growth and quality of tobacco leaves Zhao et al. (2024).

The key factor influencing the yield and quality of flue-cured tobacco are: the climate factors (including temperature, illumination and moisture) and cultivation factors (incl. transplant, fertilization, maturity and harvest) (Yang et al., 2015)

The morphological traits of tobacco plants usually depend on the genetic structure of the species and climate characteristics (Dražic, 2012; Mitreski, 2018; Kurt, 2020) and the effects resulting from the genotype x environment interaction (Butorac et al., 2004).

The interaction between genotype and environment is a universal problem that applies to all living organisms, from humans to plants to bacteria (Kang, 1998). Tobacco can adapt to a wide range of conditions but is very sensitive to the environment (Tang et al., 2020).

Dynamically changing conditions in recent years has been an additional factor for the creation of new tobacco varieties with the typical characteristics (Spasova-Apostolova et al., 2023).

The different response of genotypes to the environment is mainly related to ecology, climate and soil factors (Wu et al. 2013; Kinay, 2020). Environmental changes have a significant impact on the characteristics of tobacco genotypes (Dimanov & Zapranova, 2002) - on chemical indicators (Kalamanda & Pelivanoska, 2009; Tang et al., 2020; Kurt, 2020; Spasova-Apostolova, 2024), morphological indicators (Spasova-Apostolova, 2024; Kurt et al., 2020) and tobacco yield (Kurt et al., 2020; Tang et al., 2020).

The quality of the tobacco raw material is determined by the variety, location, environmental conditions and cultivation method (Kalamanda & Pelivanoska, 2009; Kasheva et al., 2021).

Temperature and rainfall have been found to be major factors influencing the total sugar and nicotine contents required to produce high quality Flue-cured tobacco during the tobacco growth period (Yanyan et al., 2015).

Studying the potential of tobacco genotypes in different locations and their response to environmental factors is essential to identify genotypes that are tolerant to environmental conditions.

Yield stability is an important trait of crop cultivars (Piepho, 1994). The concept of ecological stability occupies a prominent place in both fundamental and applied ecological research (Kefi et al., 2019).

The aim of the study was to evaluate the yield, morphological traits and chemical indicators of Virginia tobacco genotypes, under agroecological conditions of Plovdiv.

MATERIALS AND METHODS

The field experiment was carried out during the period 2017-2019 on meadow-cinnamon soil (Cleyic-Chromic Luvisol) (Bozinova, 2019) at experimental field (location 1) (2017; 2019) and experimental field (location 2) (2018) of the Tobacco and Tobacco Products Institute, Markovo. Three variants with parental components selected by morphological characteristics (Drumeva-Yoncheva, 2020) and resistance to TMV or PVY (Yonchev et al., 2014; Yonchev, 2014) were studied - two hybrid combinations - Hybrid 126 F_1 (Virginia 385xL 0543), Hybrid 135 F_1 (L 0543xL 0842), line L 36 F7-9 (K 326xLechia) and standard Virginia 0514. Transplanting was carried out on 29 May 2017; 22 May 2018 and 17 May 2019. The experiment was laid out in 4 replications with a harvest plot size of 27 m^2 in a 110-40/45 cm transplanting scheme. The main agrotechnical measures were carried out in accordance with the technology adopted for the cultivation of Virginia tobacco in Bulgaria.

Each genotype was analyzed for the following traits: Plant height (cm) (without the inflorescence); Number of leaves (commercial leaves); Twelfth leaf length (cm) and Twelfth leaf width (cm); Leaf area cm^2. Leaf area cm^2 calculated according to the formula: leaf length × width × 0.6345 (Suggs et al., 1960), Cured leaf yield (t/ha); chemical components - Nicotine (%), Sugars (%), Total nitrogen (%), Balance ratio total nitrogen/nicotine and sugars/nicotine. Chemical parameters were determined using AutoAnalyzer II C, Technicon in flow analyzer with methods ISO 15152:2003, ISO 15154:2003, BDS 15836:1988, respectively, under laboratory conditions.

Table 1 shows the data on meteorological conditions (values of average air temperature (ºC) and precipitation amount (l/m²) for the study period.

The main climatic indicators affecting Virginia tobacco production in the country are the average monthly temperature and the amount of rainfall. Over the three-year period of the field experiment, for the period of active tobacco growth (July and August), the average monthly temperature was higher than the norm for the region, with higher values recorded in 2017. The

three years of the study (2017, 2018, 2019) differ significantly in terms of rainfall amount. The variability of rainfall amounts across years is significant. The distribution by month during the growing season is uneven. The experimental year 2017 had the least amount of rainfall during the tobacco vegetation season compared to the following two vegetation years (Table 1).

Table 1. Meteorological data for the period 2017-2019, Plovdiv

Month	Rate	2017 (location 1)	2018 (location 2)	2019 (location 1)
Temperature, °C				
June	20.9	23.8	23.0	23.5
July	23.2	25.0	24.7	24.2
August	22.7	25.3	24.8	25.3
Rainfall, l/m^2				
June	63	15	139.9	142.3
July	49	65	82.7	38.5
August	31	9	45.6	8.7

Table 3 presents information on the reported values of the studied indicators during the three years of the experiment for the respective location.

Table 2. Soil characteristics

Year	Physical clay (%)	pH (H_2O)	Humus (%)	Total N (%)
2017, 2019 (location 1)	21	5.82	1.46	0.095
2018 (location 2)	23	6.76	1.51	0.084

The Wricke method (1962, 1966) was applied to investigate genotype-environment interaction and establish resistant tobacco genotypes:

$$W_i^2 = \sum_{j=1}^{n} (Y_{ij} - \overline{Y}_i - \overline{Y}_J + Y)^2$$

where Y_{ij} is the mean performance of genotype i in the j-th environment and $\overline{Y}_i$ is the marginal mean of the i-th genotype and $\overline{Y}_J$ is the marginal mean of the j-th genotype and Y is the overall mean.

Statistical processing of data was performed using the statistical software product IBM Statistics SPSS 24 (Field, 2013; Weinberg & Abramowitz, 2016; Landau & Everitt, 2004) и MS Excel (Mokreva et al., 2001; Pashova et al., 2025).

Table 3. Mean values for morphological and chemical indicators and the yield of Virginia flue-cured tobacco genotypes during 2017-2019

Genotype	Year	Location	Plant height (cm)	Number of leaves	Length twelfth leaf (cm)	Width twelfth leaf (cm)	Leaf area (cm^2)	Cured leaf yield (t/ha)	Nicotine (%)	Sugars (%)	Total nitrogen (%)
L 36	2017	location 1	174	28	64.0	341	1383	3.16	3.15	6.4	2.82
	2018	location 2	169	25	59.3	34.9	1310	3.06	1.07	30.1	1.25
	2019	location 1	172	21	62.6	36.7	1459	3.19	3	6.42	2.94
H 126	2017	location 1	158	27	66.6	35.1	1483	3.3	2.05	3.24	2.83
	2018	location 2	156	22	60.2	33.5	1281	3.03	0.96	23.1	1.8
	2019	location 1	158	23	66.5	37.0	1560	3.26	2.77	11.9	1.99
H 135	2017	location 1	153	25	57.1	31.7	1150	2.92	2.11	3.15	2.93
	2018	location 2	159	24	52.0	31.0	1021	2.53	0.90	29.4	1.45
	2019	location 1	154	21	60.0	35.6	1353	2.99	2.44	9.93	3.15
V 0514	2017	location 1	158	27	61.5	33.3	1300	3.12	2.17	4.52	2.61
	2018	location 2	143	21	53.0	30.3	1019	2.69	1.41	22.6	2.1
	2019	location 1	176	23	57.6	34.5	1259	2.98	1.67	15.6	2.12

RESULTS AND DISCUSSIONS

The stability of a plant variety is related to its degree of adaptability to changes in environmental factors. This enables its cultivation in areas with dynamic climatic and soil characteristics.

Plant height is an important morphological trait in tobacco. It is an indication of the uniformity of the variety. Average plant height values ranged from 143 to 176 cm over the three years of the study. Maximum mean values were measured in 2017 and 2019 at L 36 (174 cm; 172 cm) and in 2019 at standard V 0514 (176 cm) in location 1. Mitreski et al. (2018) indicates that tall-growing varieties are over 130 cm tall (Virginia and Burley). The lowest value for this trait is shown by standard V 0514 (143 cm) in location 2. Genotypes H 126 and H 135 formed shorter plants and similar height over the three-year period in both locations (Table 3). The results of the variance analysis of plant height are presented in Table 4 and Figure 1. The presence of statistically significant differences among genotypes in terms of plant height trait proved H 126 as the most resistant to environmental factors, followed by L 36.

Table 4. Comparative assessment of tobacco genotypes according to height (cm)

Genotype	Plant height (cm)	Plant height - Eco
L 36	172	8,1[b]
H 126	157	7[b]
H 135	155	32[ab]
V 0514	159	103[a]
Average	161	37.4
Std. Dev.	10.0	57.2
Sign.	0.110	0.048

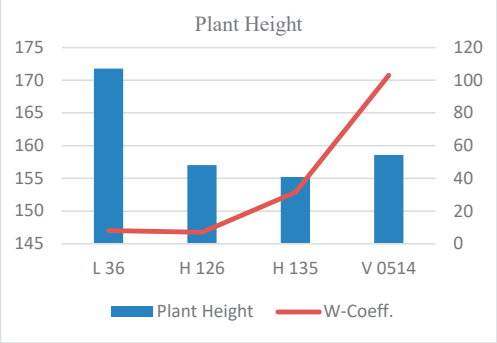


Figure 1. Stability of tobacco genotypes according to plant height (cm)

Leaf number is an essential trait determining plant productivity and is an important varietal trait (Shah et al., 2008). The number of technically suitable leaves in the studied genotypes during the three years was in the range of 21-28. In location 1, genotypes formed 21-28 number of leaves and in location 2 the number of leaves ranged from 21 to 25 (Table 3). The largest number of leaves (28) in flue-cured tobacco was reported by Zeb et al. (2023) and Nghiem et al. (2024).

In H126 and standard V 0514 the number of leaves ranged from 21 to 27, in H135 from 21 to 25 (Table 3). Shah et al. (2008) and Ahmed et al. (2014) reported maximum leaf number (25) for flue-cured tobacco genotypes.

There was no proven difference in the adaptability of genotypes for the leaf number trait over the three-year period (Table 5, Figure 2).

Table 5. Comparative assessment of tobacco genotypes according to number of leaves

Genotype	Number of leaves	Number of leaves - Eco
L 36	25	7.4
H 126	24	0.2
H 135	23	5.2
V 0514	24	0.0
Average	24	3.2
Std. Dev.	3	4.7
Sign.	0.916	0.114

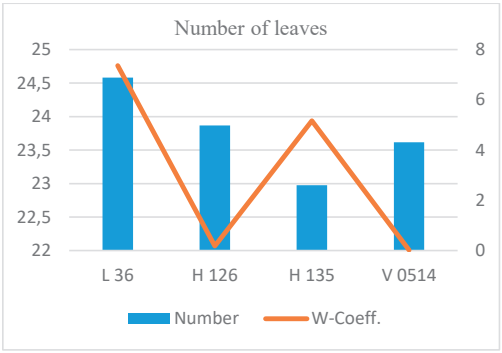


Figure 2. Stability of tobacco genotypes according to number of leaves

Leaf length and width are important morphological traits characterizing tobacco type and are the main factor influencing plant productivity. A strong positive correlation between twelfth leaf dimensions - length and width and dry tobacco yield has been demonstrated (Drumeva-Yoncheva, 2020). The relationship of leaf length and width with yield in flue-cured tobacco has also been reported by Maleki et al. (2011).

Table 3 presents the experimental data for the variants studied with respect to the twelfth leaf length trait. In the Virginia tobacco genotypes, the twelfth leaf length trait ranged from 52.0 cm to 66.6 cm over the three-year period. Mean values in location 1 ranged from 57.1 to 66.6 cm and in location 2 ranged from 52.0 to 60.2 cm.

Maximum leaf length values were recorded for H 126 (66.6 cm) and L 36 (64.0 cm) in location 1 in 2017 and 2019 and lower values in location 2. The minimum twelfth leaf lengths were H 135 and standard V 0514 in location 2.

There are statistically proven differences among genotypes for the twelfth leaf length trait. There is no proven difference with respect to the stability of the twelfth leaf length trait (Table 6, Figure 3).

Table 6. Comparative assessment of tobacco genotypes according to length twelfth leaf (cm)

Genotype	Length twelfth leaf (cm)	Length twelfth leaf - Eco
L 36	62[ab]	0.8
H 126	64[a]	0.1
H 135	56[b]	2.1
V 0514	57[ab]	1.9
Average	60.0	1.2
Std. Dev.	4.7	1.4
Sign.	0.050	0.255

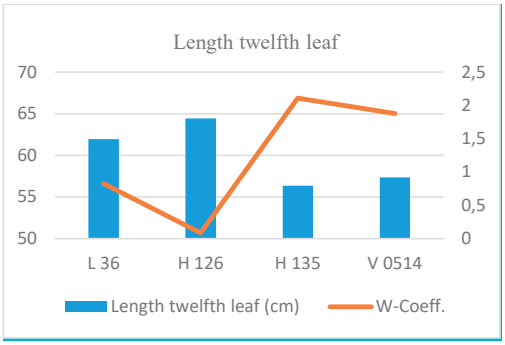


Figure 3. Stability of tobacco genotypes according to length twelfth leaf (cm)

The twelfth leaf width trait in the genotypes studied ranged from 30.3 to 37.0 cm for the three years of study. For this trait, the values obtained correspond to the values typical for the variety group of Virginia tobacco. Maximum twelfth leaf width was recorded for H 126 and for L 36 in location 1 in 2017 and 2019 (Table 3).

For the twelfth leaf width trait, there were no proven differences between the studied Virginia tobacco genotypes, both in terms of leaf size and stability of this trait (Table 7, Figure 4).

Table 7. Comparative assessment of tobacco genotypes according to width twelfth leaf (cm)

Genotype	Width twelfth leaf (cm)	Width twelfth leaf - Eco
L 36	35	0.71
H 126	35	0.04
H 135	33	0.39
V 0514	32,7	0.61
Average	34	0.43
Std. Dev.	2.12	0.44
Sign.	0.268	0.325



Figure 4. Stability of tobacco genotypes according to width twelfth leaf (cm)

Leaf area is very important component of yield in tobacco (Ahmed et al., 2016).

Data for the three years of the study on the leaf area indicator are presented in Table 3. The leaf area of the genotypes in location 1 ranged from 1150 to 1560 cm^2 and in location 2 from 1019 to 1310 cm^2. Maximum mean values for leaf area trait were recorded for H 126 (1483-1560 cm^2) and L 36 (1383-1459 cm^2) in location 1. Minimum leaf area was observed in location 2 for H 135 and V 0514, respectively (1021-1019 cm^2).

Over the three-year period, H 126 (1441 cm^2) had the highest values for the leaf area trait of the V 0514 standard and the other genotypes. Similar results were reported by Zeb et al. (2023). There were no proven differences in the leaf area trait and trait stability in the studied Virginia tobacco variants (Table 8, Figure 5).

Table 8. Comparative assessment of tobacco genotypes according to leaf area (cm^2)

Genotype	Leaf area (cm^2)	Leaf area - Eco
L 36	1384	2194
H 126	1441	198
H 135	1174	2672
V 0514	1192	2961
Average	1298	2006
Std. Dev.	169	2025
Sign.	0.108	0.371

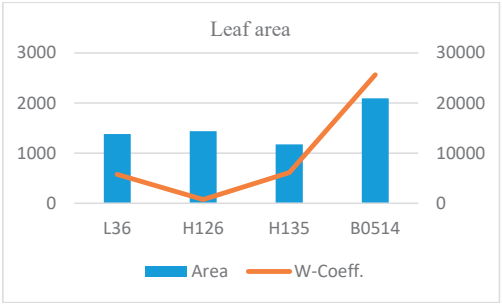


Figure 5. Stability of tobacco genotypes according to leaf area

Table 3 shows that the average values of cured leaf yield in location 1 ranged from 2.92 to 3.3 t/ha. Similar results were reported by Khan et al. (2017). In location 2, yields were in the range from 2.53 to 3.06 t/ha. Yields at L 36 and H 126 were higher and relatively stable over the three years of study. For all genotypes studied, yields were lower in location 2 in 2018. This may be due to environmental (location) conditions. Ahmed et al. (2019) summarized that tobacco genotypes were inconsistent in yield efficiency in different environments.

H 126 and L 36 were shown to form higher yields. H 135 and standard V 0514 were inferior in this indicator.
Given the values of the environmental coefficient, no line should be considered to stand out as more stable than the others (Table 9, Figure 6).

Table 9. Comparative assessment of tobacco genotypes according to cured leaf yield (t/ha)

Genotype	Cured leaf yield (t/ha)	Cured leaf yield - Eco
L 36	3.14^{ab}	0.01
H 126	3.20^{a}	0.00
H 135	2.80^{b}	0.01
V 0514	2.93^{ab}	0.00
Average	3.0	0.00
Std. Dev.	0.2	0.00
Sign.	0.051	0.305

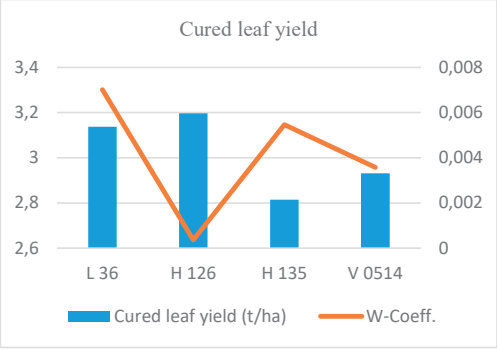


Figure 6. Stability of tobacco genotypes according to cured leaf yield (t/ha)

The content of the main chemical components nicotine %, sugars %, total nitrogen % in the genotypes was studied in 2017-2019.
Each type of tobacco within a blend has defining effects on the physical and chemical quality composition (Kurt, 2021).
There are differences in nicotine, sugars and total nitrogen content between the different years of the study (2017-2019), between the two locations and in the different genotypes.
In location 1, nicotine content varied from 1.67% (V 0514) to 3.15% (L 36), with L 36 having the maximum value. Similar results were reported by Zeb et al. (2023). At H 126 and H 135, nicotine is about 2% (Table 3). The values obtained by us are close to those indicated (Gjuzelev, 1980; Tabakova & Drachev, 1996) for a typical Virginia flue-cured (about 2-2.5%).
In location 2, where the amount of rainfall during the growing season was significant nicotine ranged from 0.9 to 1.41% (Table 3). The obtained values are close to the values indicated by Gjuzelev (1980) for Virginia produced in atypical regions – 0.6-1.2%. Hawks, 1970 assumed that high humidity contributes to lower nicotine content.
The average value of nicotine for a three-year period at L 36 (2.4%) is closest to those indicated by Tabakova & Drachev (1996) meanings of "balanced" Virginia (Table 10).
The results of the comparative assessment and ecological plasticity analysis of the tobacco genotypes showed that there were no statistically significant differences in their chemical composition (Tables 10-12, Figures 7-9).

Table 10. Comparative assessment of tobacco genotypes according to nicotine (%)

Genotype	Nicotine (%)	Nicotine - Eco
L 36	2.4	0.11
H 126	1.9	0.07
H 135	1.8	0.01
V 0514	1.8	0.21
Average	2.0	0.10
Std. Dev.	0.8	0.10
Sign.	0.787	0.209

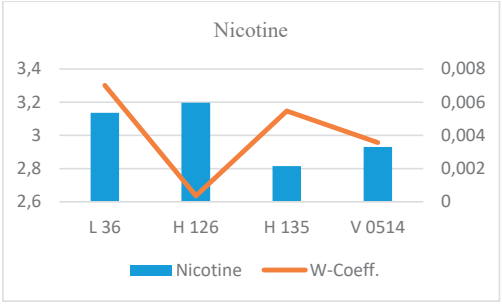


Figure 7. Stability of tobacco genotypes according to nicotine (%)

Soluble carbohydrates are one of the most important components of light tobaccos. They determine the manifestation of the characteristic pleasant taste of these tobaccos (Gjuzelev, 1980). They are generally regarded as having a positive influence on tobacco quality.
The sugar content in the 2017 and 2019 harvest samples studied was below 10%, which is atypical for the Virginia variety group tobaccos. Exceptions are H 126 and the standard V 0514 grown in location 1 in 2019, 11.9% and 15.6%, respectively. Our results are consistent with the data of Shah et al. (2008) and Zeb et al. (2023). The sugar content for the 2018 harvest is typical

for Virginia tobacco for H 126 and the V 0514 standard (Table 3).
On average over the three-year period, L 36, H 135 and V 0514 had a sugar content of 14%. In terms of the sugar content indicator, there were no statistically proven differences between the genotypes, although H 126 and H 135 stood out as more resistant to external changes compared to the other two genotypes.

Table 11. Comparative assessment of tobacco genotypes according to sugars (%)

Genotype	Sugars (%)	Sugars - Eco
L 36	14.3	12.93
H 126	12.7	2.85
H 135	14.2	3.93
V 0514	14.2	11.60
Average	13.9	7.83
Std. Dev.	10.1	8.14
Sign.	0.998	0.335

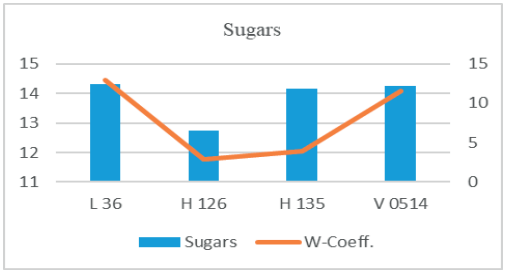


Figure 8. Stability of tobacco genotypes according to sugars

The optimal total nitrogen content for Virginia tobacco is recommended to be 1.7-2/2.3/ (Gjuzelev, 1980).
The total nitrogen content in the studied genotypes of the variety group Virginia varied widely over the three years - from 1.25 to 2.94%, with the highest nitrogen content being the samples from the 2017 and 2019 harvests, location 1, and the genotypes from the 2018 harvest having lower values (Table 3). On average for the three-year period, the genotypes with optimum total nitrogen content were H 126, V 0514 and L 36 (Table 12).

Table 12. Comparative assessment of tobacco genotypes according to total nitrogen (%)

Genotype	Total nitrogen (%)	Total nitrogen - Eco
L 36	2.34	0.10
H 126	2.21	0.10
H 135	2.51	0.11
V 0514	2.28	0.14
Average	2.33	0.11
Std. Dev.	0.63	0.08
Sign.	0.961	0.956

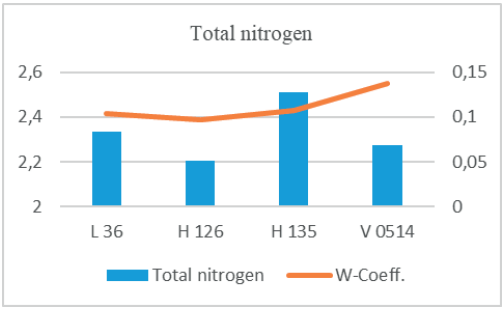


Figure 9. Stability of tobacco genotypes according to total nitrogen

When assessing the quality of tobacco based on chemical properties, the balance /ratio/ of the individual components is more decisive than the specific quantities found (Hawks, 1970). With a quantitative ratio of "total nitrogen/nicotine" < 1, the taste is well balanced (Tso, 1972). In L 36, the total nitrogen/nicotine ratio is below 1, in H 126 it is close to 1, and in the remaining genotypes it is above the permissible values.
The "sugars/nicotine" ratio provides information about the fullness and smoothness of the taste. The reference limits of this indicator are from 6 to 10.
In Table 13, the value of the sugars/nicotine ratio in the genotypes is in the range 5.68 to 8.13. The V 0514 standard demonstrates superiority for the sugars/nicotine ratio, and for H 126, H 135 and L 36 the sugars/nicotine ratio is within the reference limits according to Gjuzelev (1980).

Table 13. Balance ratio total nitrogen/nicotine and sugars/nicotine in Virginia tobacco (%)

Genotype	Balance ratio	
	Total nitrogen/ nicotine	Sugars/ nicotine
L 36	0.97	5.68
H 126	1.14	6.59
H 135	1.38	7.82
V 0514	1.29	8.13

CONCLUSIONS

The comparative evaluation carried out proves that on the traits of twelfth leaf length, cured leaf yield and plant height stability there are proven differences between the studied Virginia tobacco genotypes. On average over the three-year period, H 126 and L 36 had higher values for a twelfth leaf length, leaf area and a potential in terms of Cured leaf yield than the V 0514 standard. Based on the results of three growing

seasons, in L 36 the total nitrogen/nicotine ratio is below 1. The genotypes studied can be used in future breeding programmes.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

THE QUALITATIVE POTENTIAL OF SOME GRASS-LEGUME MIXTURES FOR FEED. CASE OF STUDY

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Abstract

The use of chemical fertilizers was one of the most common technological links for increasing feed production. The motivation for this measure was the need to increase the food requirement for the constantly growing population of humanity correlated with the consumption of animal products. One of the challenges of the last decade is to use chemical fertilizers in the smallest possible quantity, preserving the properties of the soil in order to obtain qualitative productions. One of the current trends of modern agriculture is to use mixtures (grasses and legumes) that are agro-economically efficient. In this sense, the purpose of this work is to test mixtures of annual grasses and legumes for fodder in order to evaluate their qualitative potential (CP%). Thus, mixtures of annual clover (T) and two oat varieties (O1, O2) were used. The proportions in which they were sown were: clover (T)/(O1, O2) 50%/50%, 33%/66%, 66%/33%. The mixtures were sown under the same conditions. From the statistically analyzed data, we can conclude that the best results in terms of CP% were T/O2 33%/66%, and fiber T/66%/33%.

Key words*: crud protein %, fiber %, mixture, qualitative potential.*

INTRODUCTION

In the last decade, one of the topics debated in the field of agriculture in Europe is the decline of feed quality and the use of legume mixtures with cereals with a potential to improve the agronomic performance of crop systems and to provide protein-rich foods and feed contributing to reducing European dependence on imported proteins (Watson et al., 2017; Krga et al., 2022). Reducing the use of nitrogen (N) fertilizers and their negative impact on agricultural economic activities on the environment shows that nitrogen fixing legumes cultivated in rotation or in intercalated cultures are considered a sustainable alternative to introduce nitrogen into agroecosystems (Fustec et al., 2010). Increasing the intensity of using land among small farmers is a global tendency that threatens the productive basis of farms in many developing countries.

From the perspective of environmental protection, the mixtures of legume and non-legume crops can offer more advantages than those in pure culture. Their benefits come mainly from their complex efficiency in the management of carbon and nitrogen (Kramberger et al., 2013).

Thus, the study of intercalated crops for feed and coverage crops has become a theme of interest for agronomists, biologists, and ecologists (Broker et al., 2014; Durău et al., 2024).

Annual fodder species can provide high yields and quality feed or conservation as hay or silo for subsequent feeding.

They can be cultivated as a single crop or mixed with other annual or perennial plants (Havilah, 2011).

Most research results suggest that very good performance in terms of feed quality are obtained when some annual legumes, such as *Trifolium incarnatum* L., are cultivated in mixtures (legumes - cereals) unless they are cultivated alone.

Legumes are the second most important family of agricultural crop species after grasses worldwide and are used for both cereals and feed, but are underrepresented in European crop systems, which are dominated by cereals and oil seeds.

Crimson/Italian clover grows well in mixtures with small cereals, herbs, and other species of clover.
An oat culture is a common companion, either as a coverage culture, or as a partner with high biomass and nutrients (SARE, 2012).
As Knight (1985) detailed, crimson/Italian clover can be cultivated with rye, annually ray grass, and various cereals.

MATERIALS AND METHODS

In Romania, there are few studies on the culture of crimson clover both in pure crop and in mixture for feed, maybe because of the difficulty of harvesting, keeping, and storing.
In this sense, the purpose of this paper is to highlight the qualitative potential from a fodder point of view of some crimson clover - oats mixtures.
The experimental field was located in the pedoclimatic conditions of the didactic station of the U.S.V.T. (Western Romania) in 2023 (Figure 1)



Figure 1. View from experimental field
(original foto: Carmen Claudia Durău)

Thus, the multiannual average of precipitations was, generally, between 500-650 mm, but the year 2022 was below the multiannual average (446 mm) and the year 2023 was above the multiannual average (698 mm).
The southwest of Romania, due to its geographical position in the temperate area, is exposed to a range of phenomena of meteorological and climate risk with the potential to take place throughout the year (Mircov et al., 2024).
Analysed data show that the soil is a cambic chernozem, medium clay clay/medium argyle clay with a weak alkaline pH. The concentration of humus is characteristic of this type of soil mentioned (Durău et al., 2024). The evaluation of agricultural land is one of the most important stages resulting in the increase of agricultural production and establishing a sustainable agricultural system. (Mihuț et al., 2024). The research was carried out on mixtures of crimson/(*Trifolium incarnatum* L). Kardinal (TiK) variety and two varieties of oats – (*Avena sativa* L.) Ovidiu variety (O.Ov) and Lovrin 1 variety (O.L1).
For sowing, the following mixtures were used: TIK+O.Ov 1: 1 (50%: 50%), 1: 2 (33%: 66%), 2: 1 (66%: 33%), Tik+O.L1 1: 1 (50%), 1: 2 (33%: 66%), 2: 1 (66%: 33%). Three variants were applied in three repetitions for each of the mixtures (Figure 2)



Figure 2. The experimental field
(original foto: Carmen Claudia Durău)

Each variant was 7 m^2. The mixtures were sown in the second decade of March 2023, when it was possible to enter the field.
The applied culture technology was under non-irrigation conditions and no fertilizers were applied.
According to the BBCH decimal unit for grasses and legumes (Meier, 2001), the harvesting was performed at the grass ear formation and at legume budding.
For the laboratory analyses, samples were collected from each variant on 1 m^2 and the results represent an average of the three variants. The determination of the amount of crud protein (CP%) was done by evaluating the nitrogen content using the Kjedahl method.
Statistical calculations were performed using SPSS Version 20, IBM and Microsoft Excel (2016, Microsoft Corporation, Redmond, WA, USA). Elements of descriptive statistics were determined mean, standard deviation, minimum and maximum values and 95% confidence

interval for the mean) and to determine significant differences, the Kruskal Wallis test and the Mann Whitney test were applied.

RESULTS AND DISCUSSIONS

Analysing the crud protein (CP%) depending on the combination of TiK - *Trifolium incarnatum* Kardinal and the two oat varieties in question (O.Ov and O.L1), statistically significant differences were determined in the case of the 1/2 percent (Tabel 1, Figure 3).
A similar result was obtained in an experiment carried out in the same period and in the same proportion 1: 2 (33%: 66%), but with peas and oats Lovrin 1 (Durău et al., 2024).

Table 1. Numerical characteristics associated with the mixtures according to the varieties analysed (O.Ov and O.L1) and the proportions in which the mixtures are found.

Ratio	Mixture	Mean	Std. Deviation	95% Confidence Interval for Mean		Minimum	Maximum	Mann-Whitney U(p)
				Lower Bound	Upper Bound			
Protein (CP%) 1/1	Tik+OOv	12.11	0.37	11.20	13.02	11.82	12.52	3(0.513)
	TiK+OL1	12.28	0.09	12.05	12.50	12.18	12.36	
Protein (CP%) 1/2	Tik+OOv	11.49	0.53	10.17	12.81	11.10	12.10	0(0.04<0.05)
	TiK+OL1	13.20	0.53	11.88	14.52	12.63	13.68	
Protein (CP%) 2/1	Tik+OOv	12.68	0.36	11.78	13.58	12.26	12.90	1(0.200)
	TiK+OL1	12.14	0.48	10.95	13.33	11.67	12.63	
Fiber(F%) 1/1	Tik+OO	19.64	2.15	14.30	24.98	17.87	22.03	3(0.513
	TiK+OL1	19.73	1.03	17.18	22.28	19.04	20.91	
Fiber(F%) 1/2	Tik+OO	20.32	1.02	17.79	22.84	19.61	21.48	1(0.127)
	TiK+OL1	22.24	1.97	17.33	27.14	20.16	24.09	
Fiber(F%) 2/1	Tik+OO	20.25	3.05	12.66	27.83	18.12	23.75	1(0.127)
	TiK+OL1	24.02	1.66	19.89	28.15	22.20	25.45	

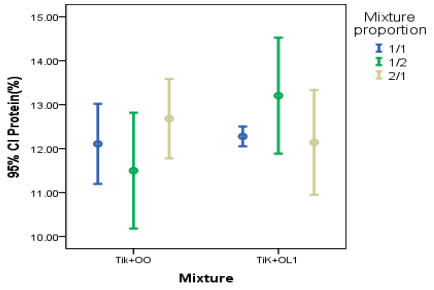


Figure 3. Mean and 95% CI for Protein (CP%)

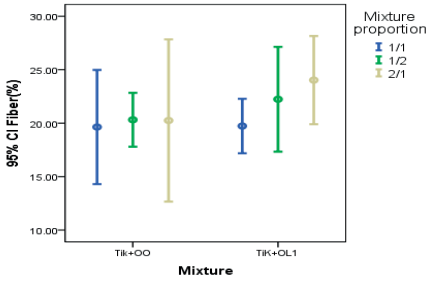


Figure 4. Mean and 95% CI for Fiber (F%)

In the case of fibre (F%), no statistically significant differences were determined between the Tik+OO and Tik+OL1 mixtures, regardless of the percentage considered (Table 1, Figure 4). Crimson clover feed is very nutritious, with over 25% gross protein that can be 80% digestible. They are harvested in early spring and can contain 12-14% raw protein and 60-65% digestible nutrients of dry substance even in full flowering (Young-Mathews, 2013; Ball et al., 2000).

Table 2. Numerical characteristics associated with the TiK+O.Ov mixture in protein (CP%) depending on the proportion

	Mean	Std. Deviation	95% Confidence Interval for Mean		Minimum	Maximum
			Lower Bound	Upper Bound		
1/1	12.11[ab]	0.36	11.19	13.01	11.82	12.52
1/2	11.49[a]	0.53	10.17	12.81	11.10	12.10
2/1	12.68[b]	0.36	11.77	13.58	12.26	12.90

Means followed by the same letter do not differ statistically (Mann-Whitney U test), *p<0.05.

Table 3. Numerical characteristics associated with the TiK+O.Ov mixture in fiber (F%) depending on the proportion

	Mean	Std. Deviation	95% Confidence Interval for Mean		Minimum	Maximum
			Lower Bound	Upper Bound		
1/1	19.63[a]	2.14	14.29	24.97	17.87	22.03
1/2	20.31[a]	1.01	17.79	22.83	19.61	21.48
2/1	20.25[a]	3.05	12.66	27.83	18.12	23.75

Means followed by the same letter do not differ statistically (Mann-Whitney U test), *p<0.05.

The analysis of the differences between the proportions in the case of crud protein (CP%) shows that there are significant differences between the proportions of 1/2 and 2/1 for the TiK+O.Ov mixture. (Table 2, Figure 5). Kramberger et al. (2013) concluded that, to obtain benefits, it is extremely important that the legumes in the mixtures of coverage or for feed be complementary to facilitate good growth under the specific conditions of the field.

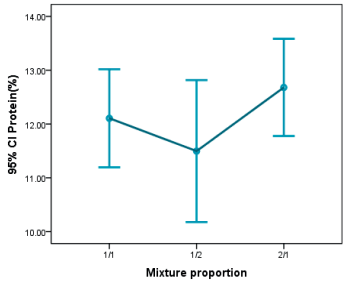


Figure 5. Mean and 95% CI for protein (CP%) TiK+O.Ov mixture depending on the proportion

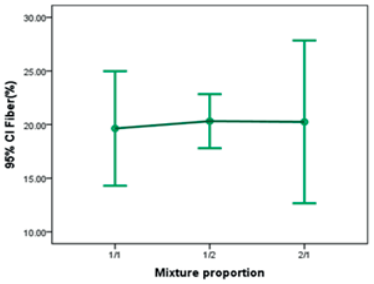


Figure 6. Mean and 95% CI for fiber (F%) TiK+O.Ov mixture depending on the proportion

For the fibre (F%) the analysis between the proportions 1/1, 1/2, 2/1 shows that there are no statistically significant differences in the case of the mixture TiK+O.Ov (Table 3, Figure 6). Analysing the percentages of crud protein (CP%) in the TiK+O.L1 mixture in relation to the proportions 1/1, 1/2, 2/1, statistically significant differences were determined between the proportion 1/1 and 1/2 (Table 4, Figure 7).

Table 4. Numerical characteristics associated with the TiK+OL1 mixture in crud protein (CP%) depending on the proportion

	Mean	Std. Deviation	95% Confidence Interval or Mean		Minimum	Maximum
			Lower Bound	Upper Bound		
1/1	12.2767[a]	0.09074	12.0513	12.5021	12.18	12.36
1/2	13.2033[b]	0.53163	11.8827	14.5240	12.63	13.68
2/1	12.1400[ab]	0.48031	10.9468	13.3332	11.67	12.63

Means followed by the same letter do not differ statistically(Mann-Whitney U test), *p<0.05.

For percentages of fiber (F %) the statistically significant differences for the TiK+OL1 mixture in relation to the percentages 1/1, 1/2, 2/1 were between the proportions 1/1 and 2/1 (Table 5, Figure 8)

Table 5. Numerical characteristics associated with the TiK+O.L1 mixture in fibre (F%) depending on the proportion

	Mean	Std. Deviation	95% Confidence Interval for Mean		Minimum	Maximum
			Lower Bound	Upper Bound		
1/1	19.73[a]	1.02	17.17	22.28	19.04	20.91
1/2	22.23[ab]	1.97	17.33	27.14	20.16	24.09
2/1	24.02[b]	1.66	19.89	28.15	22.20	25.45

Means followed by the same letter do not differ statistically (Mann-Whitney U test), *p<0.05.

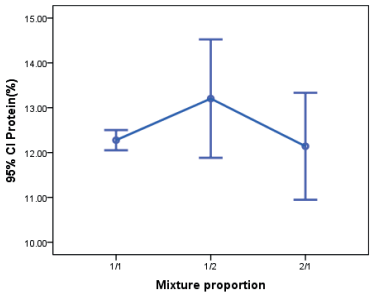


Figure 7. Mean and 95% CI or crud protein (CP%) TiK+O.L1 mixture depending on the proportion

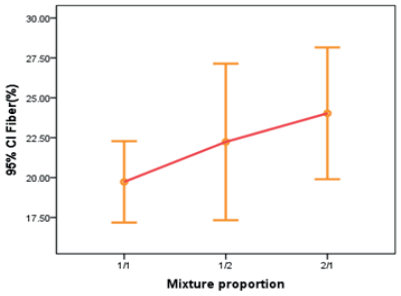


Figure 8. Mean and 95% CI for fiber (F%) TiK+O.L1 mixture depending on the proportion

As the feed matures, the fibre level increases and the protein level decreases, resulting in a general decrease in the nutritional value of the feed (Bracey et al., 2022). Akin et al. (1982) concluded, as a result of research carried out on crimson clover, that its digestibility decreases by 5% when it is harvested upon budding and 20% when it is harvested upon flowering compared to the young pre-budding stage.

CONCLUSIONS

The study of the ratios and species that fall into the composition of these fodder mixtures is very important because some species can complete each other to a certain level of participation, after which they become dominant and, implicitly, affect raw protein and fibre protein content. The interaction between the species and the moment of harvesting the mixture is very important for a maximum amount of raw protein (CP%) and fibre (F%). Data collected from the experimental field and processed with the help of Kruskal-Wallis and Mann Whitney tests have indicated some significant differences between certain parameters. Regarding raw protein (CP%), significant differences (*p <0.05) were between the two TiK+O.Ov and TiK+O.OL1 mixtures, but in the same proportions 1: 2 (33%: 66%) where oats O.OL1 also used the resources better. The largest amount of fibre (F%) was obtained with TiK+O.OL1 (F%-24) mixture but no significant differences were found compared to the TiK+O.Ov mixture, for the same ratio. Significant differences in the case of raw protein (CP%) were found for the same mixture but in different proportions 1: 2 (33%: 66%) and 2: 1 (66%: 33%) for TiK+O.OL1 1: 1 (50%) and 1: 2 (33%: 66%) TIK+O.Ov, respectively. This case study is for a short period of time and there were obviously other factors that contributed to the outcomes. The research will continue for a longer period to highlight the benefits and qualitative potential of the mixture of oats with crimson clover under the conditions of Timiș County (western Romania).

ACKNOWLEDGEMENTS

This paper is published from the own research funds of the University of Life Sciences "King Mihai I" from Timisoara.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

COMPARATIVE TESTING OF SORGHUM, SUDANGRASS AND MAIZE SOWN AS A SECOND CROP AFTER WHEAT AND FALLOW PREDECESSORS

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Abstract

The research was carried out between 2021 and 2023 in the experimental fields of the Agricultural Institute - Shumen, Bulgaria. The aim of this study was to establish the yield structure and productivity of grain sorghum, Sudangrass and grain maize tested as a second crop after wheat and fallow predecessors. The results over three consecutive years indicate that fallow is a better predecessor and the plants sown on it develop much more intensively and accumulate more mass than the wheat predecessor, despite the shorter vegetation periods. The green mass yields of Sudangrass, sown on fallow significantly exceeded the yields obtained with the wheat predecessors. Similar results were obtained with sorghum and maize. In all three years, maize and sorghum with a fallow predecessor managed to form seeds and reach the milky-wax maturity stage, which is an important prerequisite for the production of green silage. Crops sown on fallow, despite their early sowing, lag behind in growth and form only short stems and foliage.

Key words*: sorghum, Sudangrass, maize, productivity, predecessors.*

INTRODUCTION

Growing more and better quality produce is the goal of any agricultural production. For summer cash crops and for those grown for green manure after cereal crops in the conditions of Northern Bulgaria, precipitation is insufficient, especially after a predecessor that uses a large part of the water accumulated in the soil from autumn-winter precipitation. Often after harvesting the wheat crop, the productive moisture in the 0-25 cm layer is exhausted. When maintaining fallow (for the implementation of the administrative measures under Regulation (EU) 2021/2115 of the European Parliament and of the Council of December 2, 2021, it is necessary that the plot is in good agricultural condition by July 15), the soil is moisture-retained and enriched with easily accessible nutrients. Sowing summer crops for the purpose of obtaining cash produce, fodder or for green manure has been a widespread practice for centuries. The problems facing such production are not few. On the one hand, the relatively short growing season does not allow crops to develop their potential, and on the other hand, plant development in the driest and hottest months is a challenge. The search for crops with a short growing season and high yield potential under stressful conditions requires efforts to be directed towards better understanding their genetic and physiological capabilities in order to use them in breeding. Also, the environmental standards that have been imposed administratively in recent years require improved field management. Intercrops are a key component of the cropping sequence in minimum tillage farming systems. They allow for minimizing the accumulation of diseases and the rotation of herbicide groups to combat herbicide-resistant weeds (Kirkegaard et al., 2008; Angus et al., 2015; Kirkegaard & van Rees, 2019).

Integrating cover crops into cropping systems has costs and benefits, both internal and external to the farm. Benefits include promoting pest suppression, soil and water quality, nutrient cycling efficiency, and main crop productivity. Costs of growing cover crops include increased direct costs, potentially reduced income if green manures interfere with other attractive crops, difficulties in predicting nitrogen mineralization, and production costs. The benefits of cover crops are generally higher in irrigated systems (Snapp et al., 2005). Crop production systems in semi-arid regions may not have the potential to profitably use cover crops due to reduced subsequent yields of wheat

(*Triticum aestivum* L.) after the cover crop. There is no evidence of yield reduction for subsequent spring crops (Nielsen et al., 2016).

Increasing soil organic carbon is easiest with cover crops in cropping systems. Their advantage over other management practices is that they do not cause yield losses, such as extensification, nor carbon losses in other systems, such as organic fertilizer applications. However, the effect of green manuring of cover crops on soil carbon stocks has been widely neglected (Poeplau and Don, 2015).

Cover crops are a more reliable means of increasing main crop yields than fallow and produce the highest amount of biomass and should be considered when the goal is to quickly build soil organic matter (Snapp et al., 2005).

In low-rainfall environments, high frequency of winter cereals is preferred to optimize productivity and risk. However, high-intensity cereal crops often result in reduced water use efficiency and increased inputs to address emerging nutritional, disease and weed problems. Incorporating green manures into subsequent cereal crops has been shown to increase yields by at least 1 t ha across a range of soil types and has beneficial effects on nutrient cycling and delivery, along with some short-term effects on *Rhizoctonia solani* infection in subsequent cereal crops, while there is no convincing evidence of improved water balance for subsequent crops (McBeath et al., 2015). Han et al. (2021) found a difference in the accumulation of all types of nutrients in the topsoil from lower soil horizons, as well as increased uptake by the subsequent crop. This also helps to conserve nitrogen from denitrification. The obtained data on grain yields and fertilizer application indicate the importance of the formation of a mulching layer of plant residues on the surface of ordinary and southern chernozems in no-tillage systems. This layer contributes to the gradual accumulation of nitrogen, protects the soil from erosion, and increases the number and mass of combthworms. Nitrogen in direct-tillage systems on chernozems in Russia is characterized by high mobility and the ability to undergo significant transformation. In this context, additional research and special experiments are needed to obtain better knowledge of the behaviour of nitrogen in different cropping systems. In particular, the relationships between nitrogen flows, agrophysical properties of the soil (the distribution of aggregates by size at the macro and micro levels) and the specificity of organic matter in different subtypes of chernozems under different cultivation technologies need to be clarified. In general, the role of direct-tillage systems in agricultural practices has become more significant in recent years. The application of the system of cultivation of crops with minimal and zero tillage is considered a positive factor for maintaining and restoring soil fertility (Zavalin et al., 2018). Not all crops are suitable for cultivation in summer sowing.

C_4 and C_4-like plants have higher water use efficiency than C_3 plants (Tanigawa et al., 2024). They are equipped with a CO_2-concentrating mechanism that mitigates photorespiration (the wasteful Rubisco process of fixing O_2 instead of CO_2) and improves CO_2 assimilation (Leegood, 2002; Keely and Rundel, 2003).

Crops with C_4 photosynthesis are exceptionally suitable for summer sowing and suppress plant parasitic nematodes and soil-borne diseases (Snapp et al., 2005).

Analysis of the productivity of grain maize, grain sorghum and Sudan grass sown after wheat harvest under different climatic conditions contributes to better planning and management of farms. The goal is to study typical crops with C_4 type of photosynthesis because they better utilize moisture during extreme droughts and high temperatures, and provide reliable yields.

MATERIALS AND METHODS

The experiment was conducted on the experimental fields of AI - Shumen in 2021-2023. The area in which the experiment was conducted is located in the zone of typical temperate continental climate. It is characterized by cold winters and hot summers. The relief is flat. The soils are typical and leached chernozems. The average annual precipitation is about 620 mm. The distribution is very uneven. Usually the maximum precipitation is in May and June. This is very important, because this allows for the successful development of field crops sown in the fall. The soil nutrient supply

is moderate with a humus content of 3.4%, N - 48 mg/kg, P_2O_5 - 5.7 mg/100 g., K_2O - 134 mg/100 g, B - 1.3 mg/100 kg, Mn - 45 mg/kg., Zn - 0.1 mg/kg, Mo - 0.1 mg/kg. After wheat, the necessary amounts of NH_4HPO_4, KNO_3 and NH_4NO_3 were applied to compensate for the nutrients extracted from the crop. Water-soluble fertilizers were selected to quickly compensate for the losses. The fields for the experiment over the years were different, grouped in one array. The predecessor of wheat for all three years was corn. The treatments for the predecessor included three treatments with a disk harrow in different directions and the application of fertilizers before sowing. Sowing was carried out at a depth of 3-4 cm with subsequent rolling with light articulated rollers. The wheat was sown in combly October in the year preceding the sowing of the catch crops. Since we do not believe that the sowing dates of the predecessor have an impact on the experiment, the exact dates are not indicated. For the needs of the experimental setup, the following crops were selected:

1. Hybrid grain corn PR 9400;
2. Grain sorghum - Maxired variety;
3. Sudan grass - Enje-1 variety.

The experiment was carried out in two variants:
Variant 1. Sowing of grain corn, grain sorghum and Sudan grass immediately after harvesting the predecessor wheat
Variant 2. Sowing of grain corn, grain sorghum and Sudan grass on a cultivated field with a predecessor fallow.
In variant 1 (predecessor wheat), the area was kept free of weeds by vegetative treatment with herbicides. Special attention was paid to their selection so that they did not affect subsequent crops, due to the very short time from application to sowing. In addition, the crop affects weeding through competition for nutrients, water and light.
In variant 2 (predecessor fallow), the area was kept clean by cultivation. The time for mechanical processing was adjusted to be immediately after rain in order to preserve the accumulated moisture.
The mass and germination of 1000 pcs. seeds for each of the three crops, according to the pleated filter paper method in a thermostat at 26°C.
The field experiment was based on the long plot method. Each variant was on an area of 100 m^2, with each crop occupying 25% of the area. Sowing was done manually at a depth of 6 cm for grain maize and 4 cm for Sudan grass and grain sorghum.
After the predecessor wheat, in different years the sowing was carried out on different dates, depending on its harvesting period. In 2021, the harvest was on June 28, with the sowing of the intermediate crops being carried out on July 1st. In 2022, the predecessor was harvested on July 3rd, and the sowing was carried out on July 4th. In 2023, the predecessor was harvested on July 5th, and the sowing of the intermediate crops was carried out on July 6th. When sowing, we made efforts to reach a moist layer to allow the seeds to germinate with the moisture available in the soil and to mulch the rows with straw as much as possible. The sowing rates of the tested crops are as follows: corn for grain - 65,000 seeds per hectare, sorghum for grain 350,000 seeds per hectare, Sudan grass 400,000 seeds per hectare. The seeds and the experimental plots were tested for the presence of phytopathogens and enemies. Due to their absence, chemical disinfection of the seeds for sowing was not carried out. During the growing season, no serious attacks from diseases and enemies were observed, probably due to the uncharacteristic period of development, as well as the dry and hot weather. Weed control was carried out by hand weeding. The green mass was harvested at the beginning of October, immediately before the first autumn frosts. The composition of the yield of fresh and dry mass was determined and an analysis of the components of the yield was performed - stems, leaves, panicles, combs.
The aim of the study is to establish the yield structure and productivity of grain sorghum,
Sudan grass and grain maize tested as a second crop after predecessors wheat and fallow.

RESULTS AND DISCUSSIONS

The results shown in Table 1 show the mass and germination of seeds from the three crops.
It is evident that the weight of sorghum seeds, due to their small size, is almost 1:10 compared to corn seeds, and Sudan grass 1:16. Accordingly, 340 g: 21.6 g. The mass of the seeds is within normal limits for the species and determines a reserve of nutrients available to the plants after germination. The germination of all

three crops is excellent with an average germination of over 95%, with Sudan grass being lower. This is especially important when determining the sowing rates of the crops.

Table 1. Mass and germination of seeds of grain maize, grain sorghum and Sudan grass

Grain maize		Sudan grass		Grain sorghum	
Mass (g)	Germination rate (%)	Mass (g)	Germination rate (%)	Mass (g)	Germination rate (%)
340.4	98.0	20.8	95.0	36.6	96.0
341.2	99.0	22.0	96.0	36.0	98.0
340.6	98.0	22.2	95.0	36.0	97.0
340.7	98.3	21.6	95.3	36.2	97.0

Table 2 shows the precipitation and temperatures during the testing period, and for better illustration, previous months are also included, since the precipitation in them is outside the vegetation period of the experiments, they create a reserve of moisture in the soil, which can subsequently be used during the vegetation period.

Meteorological data during all three years of conducting the experiments indicate an increasing water deficit and a tendency towards extreme deviations from agroclimatic norms. The annual precipitation for all three years is around the average norm for the region, with 2022 even slightly exceeding the norm, but for the three months of vegetation they are in acute shortage. 2021 is distinguished by less precipitation than 2022, but due to their more correct distribution at the beginning of the period, the crops showed the highest results. 2023 is the most unfavourable year in terms of moisture. On the one hand, the precipitation that fell was far below the norm, and on the other hand, a large part of it was at small due to the short day, low temperatures and fading vegetation. During the three years of testing, the crops actually sprouted normally and developed with moisture accumulated as a reserve in the soil. Then they slowed down their growth, and this was very characteristically seen in the crops sown after a predecessor of wheat, practically stopping their growth. With a predecessor of fallow, the growth slowdown was not so noticeable and the crops developed much better. The high average temperatures in the region also contributed to the stress on the plants. The second and third ten days of July, as well as the beginning of August, were very hot in all three years of testing, with average daily temperatures of 24-26°C. The record for the three years was the second ten days of July 2023, with an average temperature of 27.5°C. This led to a delay in crop development.

Table 2. Meteorological data for the experimental area

Years	Month	Rainfalls, mm					Temperature			
		Decades			Amount	Norm	Decades			Average
		I	II	III			I	II	III	
2021	VI	16.7	136.4	11.7	164.8	41	17.6	17.7	25.3	20.2
	VII	16.5	3.4	1.2	21.1	64	18.9	22.5	26.7	23.4
	VIII	0	0	6.7	6.7	42	24.6	25.4	22.5	24.1
	IX	0	1.7	1.4	3.1	53	18.5	21	15.3	18.3
Total for the period					290.8	305				
Years	Month	Rainfalls-mm					Temperature			
		Decades			Amount	Norm*	Decades			Average
		I	II	III			I	II	III	
2022	VI	52.7	73.3	1.2	127.2	41	21.2	20.2	22.6	21.3
	VII	0.6	0	3.7	4.3	64	23.5	22.9	26.1	24.2
	VIII	0	27.6	3	30.6	42	25.1	24.4	24.8	24.8
	IX	28.9	9.6	16.7	55.2	53	20	19	16.2	18.4
Total for the period					309.1	305				
Years	Month	Rainfalls-mm					Temperature			
		Decades			Amount	Norm	Decades			Average
		I	II	III			I	II	III	
2023	VI	13.3	11.4	44.7	69.4	64	13.8	15	17.9	15.6
	VII	34.6	3.1	0	37.7	64	23.8	27.5	25.3	25.5
	VIII	0	6.7	0.6	7.3	42	24	24.9	20.8	23.1
	IX	0.9	47.8	0	48.7	53	22	20.4	21.3	21.2
Total for the period					275	305				

*mean of vegetation rainfalls for 50 years period

After germination, despite the initial stress caused by drought, the crops managed to accumulate vegetative mass in the initial stage of their development. Grain maize, due to its larger seeds and, accordingly, the larger supply of nutrients in the endosperm, logically overtook grain sorghum and Sudan grass in the initial stages. It quickly accumulated biomass and grew in height. An important detail is that on a field with a fallow predecessor during the three years under consideration, grain maize failed to reach comb development, but managed to develop only leaf mass. Drought and high temperatures led to typical physiological

features - strong shortening of the internodes, shrinkage of motor cells and curling of the leaves, reduction in chlorophyll content. On a field with a fallow predecessor, the development of grain corn was accelerated and not only were the plants taller, but they were also able to set combs, and in all three years of the experiment the crop reached milky-wax maturity of the grain. The physiological changes were not so well expressed. The stems reached a length characteristic of the variety and the comb formation, flowering and pollination proceeded normally.

Grain sorghum quickly sprouted in both predecessors. However, we can note that in the predecessor fallow, it quickly accumulated biomass and grew in height, reaching the height and thickness of the stem normal for the variety, with the phases of sweeping, flowering and pollination occurring normally. At the end of the experiment, it reached the flowering phase - milky-wax maturity in all three years. On the predecessor, wheat reached the development of only leaf mass, with strongly shortened internodes with insignificant stem height, and only in 2021 did it manage to reach sweeping, but the panicles remained sterile.

In Sudan grass, we found similar data to grain sorghum. In the predecessor fallow, although with a delay in sowing until July 15, it sprouted normally in 3-4 days. It quickly caught up with that sown on stubble and grew in height, reaching a height of 2 m in 2021, and at the end of the growing season it was in the sweeping phase. In the following years, the effect was almost the same. On the predecessor wheat, after normal germination and topping of the crops and reaching the 2nd, 3rd leaf phase, its growth slowed down significantly. By the onset of autumn, it managed to make 5-6 leaves with greatly shortened internodes on the stem. The plants were severely stressed and with a reduced chlorophyll content.

The average data on green mass yield are shown in Table 3.

As can be seen from the table, 2021 is distinguished by higher green mass yields than the following two for all crops. Despite the relatively lower precipitation compared to 2022, they are grouped at the beginning of the period and create a reserve of moisture, which was subsequently utilized by the plants. In addition, 2021 is the coolest of the three years of testing and with the lowest evapotranspiration coefficient. Corn yields on fallow exceed those of 2022 by 0.514 t/ha or 2% and those obtained in 2023 by 1.755 t/ha or 6%. This trend is also maintained for the predecessor wheat stubble, where the difference is 0.908 t/ha or 14% and 1.563 t/ha or 27%. For Sudan grass rye, the decrease on fallow for 2022 and is 11,250 t/ha or 21% and 13.25 t/ha or 26% for 2023, and on stubble 1,185 t/ha or 37% for 2022 and 1.2 t/ha or 38% for 2023. For sorghum on fallow, the yield reduction in 2022 and 2023 is respectively 6 t/ha or 22%, and 8 t/ha or 28% for 2023, and on predecessor wheat 0.65 t/ha or 25%, and 0.43 t/ha or 17%.

Table 3. Total yield of green mass of crops sown after predecessors fallow and wheat (t/ha)

Year	Grain maize yield (t/ha)		Sudan grass yield (t/ha)		Grain sorghum yield (t/ha)	
	Fallow	Wheat	Fallow	Wheat	Fallow	Wheat
2021	30.87	5.87	52.30	3.21	28.85	2.65
2022	30.36	4.96	41.71	2.03	22.70	1.99
2023	29.11	4.31	39.10	2.01	20.81	2.22
Mean	30.11	5.05	44.38	2.41	24.12	2.28
GD1 %	6.058	5.0	9.34	4.04	4.56	9.13
P%	5.2	16.1	6.45	3.45	5.61	3.12

The differences between the yields after the different predecessors are greater. Maize harvested on stubble in 2021 gives only 19% of the yield on fallow. In 2022, 16%, and in 2023 only 14.8%. On average for the three years of testing, the reduction is 25 t/ha. In Sudan grass, the different predecessors have a stronger influence. In 2021, the yield obtained after the predecessor wheat is only 6.1% of the yield obtained after the predecessor fallow, with the difference being 49 t/ha, and in 2022 and 2023, respectively 4.8 or 39.5 t/ha and 5.1% or 37 t/ha. In sorghum, the yields obtained with wheat as the predecessor are respectively 9% or 26 t/ha, 8.7% or 20.5 t/ha and 10.6% or 18.5 t/ha of those obtained with fallow as the predecessor. The strongest influence of the predecessor on the difference in yield is found in Sudan grass. Due to the small seeds and the few reserve nutrients in the endosperm, it germinates and develops extremely slowly on stubble, being strongly oppressed by drought and high

temperatures. The difference in yield over the years between fallow and stubble reaches 18 times, with the largest differences being found in 2021.

Table 4. Analysis of yield components for grain maize, grain sorghum and Sudan grass with a fallow predecessor

Year	Fallow predecessor - total yield of green mass (t/ha)								
	Grain maize			Sudan grass			Grain sorghum		
	Stalks	Leaves	Cobs	Stalks	Leaves	Panicles	Stalks	Leaves	Panicles
2021	19.382	9.825	1.666	41.080	10.752	0.517	20.035	5.810	3.005
2022	20.530	8.700	1.130	30.440	10.925	0.347	15.767	4.572	2.365
2023	19.580	8.266	1.273	28.785	9.615	0.700	13.452	3.991	2.167
Mean	19.830	8.930	1.356	33.435	10.430	0.521	16.751	4.858	2.512
GD1%	5.941	5.043	0.565	4.248	1.678	0.396	3.509	1018	0.565
P%	6.52	13.42	9.33	2.76	3.5	16.51	4.56	4.13	3.21

In corn, the composition of the yield with a fallow predecessor did not differ significantly during the three years of testing. In 2021, with a higher total yield, the percentage of stems in the total yield was 62%, leaves 32%, and combs 5%. In the following two years, the share of stems increased by 5% to 67%, while leaves decreased by 5% and combs by 1%. In Sudan grass, the trend was the opposite. The share of stems was the largest in 2021, 78%, in the following two years it made up about 73%, this is due to the increase in leaf mass in 2022 and 2023 to 26% and 25% and almost no change in panicles. The composition of the yield in sorghum almost did not change during the tested years. The share of stems in 2021 was the largest, making up over 70% of the yield. In the following two years it drops to 69% and 68%. The share of the yield taken up by the leaves is 19.4% in 2021 and increases to 20% in the following two. In the case of cornflowers, the relative yield in 2021 and 2022 is 10% of the total mass, and in 2023 11% (Table 4).

The yield composition of the predecessor wheat in all three crops consists exclusively of leaves. This is due to the low height of the plants and the very shortened stem during harvesting. The highest yields of corn were obtained in 2021 - 5.875 t/ha., and the lowest in 2023 - 4.312 t/ha When reviewing the data on the Sudan grass yield, it is striking that the highest was obtained in 2021 - 3.215 t/ha, and the lowest in 2022 - 2.032 t/ha. The yield of grain sorghum varies from 2.65 t/ha for 2021 to 2 t/ha for 2022, with the smallest variation in productivity being found in this crop (Table 5).

Table 5. Green mass yield with wheat as predecessor (t/ha)

Years	Wheat predecessor - total yield of green mass (t/ha)		
	Grain maize	Sudan grass	Grain sorghum
2021	5.875	3.215	2.650
2022	4.967	2.032	1.998
2023	4.312	2.257	2.220
Mean	5.052	2.336	2.298
GD1%	2.745	0.323	0.563
P%	11.82	12.33	5.35

Table 6. Green mass of post-harvest residues for different predecessors (t/ha)

Year	Post-harvest residues in a predecessor fallow (t/ha)			Post-harvest residues in a predecessor wheat (t/ha)		
	Maize	Sudan grass	Grain sorghum	Maize	Sudan grass	Grain sorghum
2021	10.810	13.905	6.185	2.875	3.105	4.393
2022	8.575	12.730	5.717	2.465	2.448	3.948
2023	9.325	10.610	5.935	1.975	2.258	3.424
Mean	9.903	12.415	5.945	2.439	2.604	3.922
GD1 %	5.685	2.490	0.580	0.652	1.032	0.371
P%	13.5	4.32	3.2	5.82	8.62	2.06

From the three years of harvesting corn after a predecessor fallow, an average of 9.900 t/ha. post-harvest residues were obtained. The most in 2021 – 10.810 t/ha., and the least in 2022 – 8.575 t/ha, with a significant variation. They constitute 25% and 23% of the total yield, respectively. Sudan grass with the same predecessor gave the most post-harvest residues in 2021, and the least in 2023, respectively 13.90 t/ha and 10.61 t/ha., varying from 21% to 24% of the total yield. In sorghum, post-harvest residues vary relatively little, between 5.717 t/ha in 2022 to 6.185 t/ha in 2021. It constitutes 17% and 21% of the total yield, respectively.

With wheat stubble as the predecessor, the amount of post-harvest residues in maize is between 2.875 t/ha and 1.975 t/ha, with the

weight ratio between the obtained yield and plant residues being from 46% to 49%. In Sudan grass, their amount is less than that in maize and sorghum, with their amount varying from 2.010 t/ha to 3.105 t/ha. The percentage ratio is similar to maize - 49% to 54%. The yield in sorghum is from 2.000 t/ha to 2.650 t/ha, with the percentage ratio plant residues/yield being between 60% and 66%.

In 2021, Sudan grass recorded the highest amount of plant residues - 3.105 t/ha, and the lowest in 2023 - 2.258 t/ha. The highest amount of plant residues after the predecessor wheat was recorded for grain sorghum. Yields are relatively constant with little variation. Expressed in absolute values, they range from 4.393 t/ha in 2021 to 3.4224 t/ha in 2023 (Table 6). This is due to the relatively thick stems of the plants in their lower part and the high sowing rate.

CONCLUSIONS

Sowing corn, sorghum and Sudan grass as a second crop for green fodder or for green manure after fallow is appropriate in view of the unfavorable agroclimatic conditions caused by drought. The obtained average yields of 30 t/ha corn, 44 t/ha Sudan grass and 24 t/ha sorghum confirm the possibility of their use in agricultural production.

The yields obtained from the three crops after the predecessor wheat in the three years of conducting the experiment are unsatisfactory and in the presence of droughts question their use in practice as a second crop.

The obtained green mass yields with the predecessor fallow significantly exceed those obtained with the predecessor stubble. The increase in corn on fallow is 25 t/ha, with Sudan grass 42 t/ha, and with sorghum 22 t/ha.

The amounts of plant residues obtained with the predecessor fallow are superior to those obtained with the predecessor wheat. The differences in the average amount for corn are 7.5 t/ha, for Sudan grass 12 t/ha, and for sorghum 2 t/ha.

For the predecessor wheat, the yields are insignificant, with post-harvest residues representing the majority of the biomass obtained.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

EFFICACY OF BIOLOGICAL AND CHEMICAL SEED TREATMENTS FOR SUSTAINABLE MAIZE CROP PROTECTION

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Abstract

This study evaluates the efficacy of maize seed treatments to identify environmentally friendly crop protection solutions and reduce reliance on toxic chemical pesticides. Conventional products (Signal, Redigo Pro) and organic treatments (Bioseed, Biosem, Raiza Mix) were tested during 2024 at ARDS Turda (Transylvanian Plateau) and ARDS Brăila (Romanian Plain). Requiem Prime and Repel Aves, typically used as foliar repellents, were experimentally tested as seed treatments. The experiments were conducted on Turda 344 and Harmonium hybrids, respectively, analyzing seedling emergence dynamics, pest resistance, yield, hectoliter weight, and 1000-kernel weight. At ARDS Turda, Repel Aves (250 ppm) showed the highest emergence and yield increases (20-25%) under drought conditions, while higher concentrations (1000 ppm) caused phytotoxicity. At ARDS Brăila, Requiem Prime (250 ppm), Redigo Pro, and Signal achieved the best results, with high yields and superior quality. Organic treatments like Bioseed and Biosem showed intermediate performance, while untreated seeds had the weakest results. The study highlights biological seed treatments as effective and sustainable alternatives, enhancing yield and resilience to climatic stressors while supporting eco-friendly agriculture.

Key words*: biological treatments, climatic conditions, seed treatment, sustainable agriculture, yield.*

INTRODUCTION

Romania's temperate-continental climate and nutrient-rich soils create favorable conditions for agriculture, making it a vital sector in many parts of the country. Maize stands out as the dominant grain crop, in terms of cultivated area and overall production. Protecting crops against pests and abiotic stress represents a major challenge for farmers, especially in the context of climate change and the need to reduce the use of conventional pesticides. In this context, innovative seed treatments based on diverse active substances are essential to ensure optimal crop development and to reduce losses caused by pathogens and pests.

Given this, testing commercial and experimental products with insecto-fungicidal effects has become a priority. Field experiments began with the evaluation of commercial products with bird-repellent effects, which were initially used for treating crops during vegetation rather than for seed treatment. The main reason for this research direction was to identify a solution to the problem of biologically treated maize seeds, which birds often consume before they have a chance to germinate (Georgescu et al., 2024).

Methyl anthranilate is a natural aromatic compound recognized for its repellent effect against birds and insects (Mason et al., 1989; Avery & Decker, 1994; Avery et al., 1995; Mason & Clark, 1995; Blackwell et al., 2001). According to research conducted by Avery et al. (1998), this substance has the ability to deter birds from consuming seeds, thereby protecting crops during the early stages of development.

Products based on organic substances and plant extracts have gained popularity as a sustainable alternative to conventional pesticides. Terpenes act as natural repellents (Plata-Rueda, 2020). They work by interfering with the olfactory system of insects, disrupting their ability to

detect hosts or food sources. The strong aroma of these compounds masks attractive chemical signals or produces a disorienting effect, causing insects to avoid treated areas (Boncan et al., 2020). Tabanca et al. (2013) highlighted that the terpenoid p-Cymene, when used alongside other terpene compounds, demonstrated high efficiency in repelling phytophagous insects such as mosquitoes and grain beetles.

In Romania, most commercial products for treating maize, sunflower, wheat/autumn cereals, and rapeseed seeds against diseases and pests are chemically synthesized. These include: pyrethroid insecticides designed to combat soil pests (e.g., *Agriotes* spp., *Melolontha melolontha, Tanymecus dilatic*ollis, etc.), fungicides for cereal seed treatment targeting pathogens such as *Cochliobolus* sp., *Fusarium* sp., *Septoria* sp., *Tilletia* sp., *Ustilago* sp., etc., insecto-fungicides formulated as concentrated microcapsule suspensions.

Combined fungicides that contain two active substances with different modes of action and activity spectrum.

These products can be either non-systemic or systemic, contact-based, or repellent, with limited assimilation and translocation of the active substance within the plant.

Only a few biological seed treatment products are available on the market, and most are based on plant extracts or mixtures containing neem oil, organic matter, microorganisms, humic acids, and fulvic acids.

The objective of these experiments was to evaluate the efficiency of various seed treatments applied to maize seeds in order to protect crops and optimize their yield under different agro-pedoclimatic conditions.

MATERIALS AND METHODS

The research was conducted in 2024 at A.R.D.S. Turda and A.R.D.S. Brăila, located in two distinct agricultural regions, each with different soil and climatic conditions. This allowed for a detailed comparative evaluation of the effectiveness of the treatments applied to the maize crop.

At A.R.D.S. Turda, situated in the Transylvanian Plain, the soil is classified as vertic clay-illuvial soil, with a loamy-clay texture, neutral pH, and good to very good phosphorus and potassium availability. The soil has a medium humus content and is prone to rapid compaction under high moisture conditions or the action of heavy machinery.

In this context, the research was conducted under climatic conditions characterized by high temperatures and severe drought. The 2024 agricultural year recorded significant temperature deviations from the multiannual average, and a major rainfall deficit, particularly in June (-48.4 mm below the multiannual average), negatively impacted crop development.

In the Turda experiment, the Turda 344 hybrid was used, with a sowing density of 57,000 plants/ha.

Plant emergence was monitored at three different intervals:

- 10 days after sowing (14.05.2024),
- 24.05.2024, and
- 05.06.2024.

Simultaneously, assessments were conducted on damage caused by pests and diseases, including wireworms (*Agriotes* sp.), crows, cutworms (*Agrotis segetum*), and rotting plants.

The harvested yield was evaluated in kg/ha. The experiments conducted at A.R.D.S. Brăila aimed to achieve the same objectives but under different agro-pedoclimatic conditions. The predominant soil type in this area is a cambic chernozem, characterized by high fertility but prone to rapid moisture loss.

The 2024 climate in this region was marked by extreme temperatures and severe rainfall deficits. Although total precipitation reached 471 mm, slightly above the multiannual average, its uneven distribution required the implementation of special strategies to protect crops from water stress.

In this experiment, the Harmonium hybrid was used, with the same sowing density of 57,000 plants/ha as in the Turda experiment. The treatment variants applied were identical to those used at Turda.

At Brăila, parameters related to plant emergence and pest or disease attacks were not monitored. The evaluation focused exclusively on the following parameters:

- Number of cobs/10 m²;
- Harvest moisture content (U%);
- Hectoliter mass (HM);
- Thousand Grains Weight (TGW, g);

- Grain yield (%) (the ratio of kernel weight to total ear weight, excluding the cob - rachis);
- Production (kg/ha).

The products used in the applied treatments. For seed treatment and stimulation of maize crop development, several market-available products with different roles were used: a fungicide based on cypermethrin 300 g/L (Signal 300 ES) applied at 2 L/t, a biostimulant based on plant extracts and beneficial microorganisms (Raiza mix) applied at 2 L/t. To protect roots against diseases and promote soil health, the product Mycoforce was used at 100 g/t, a mixture containing mycorrhizal fungi, *Trichoderma* sp., and *Bacillus* sp.

The experiment also included products based on organic substances and plant extracts. Requiem Prime, with the active substance containing 152.3 g/L of a terpenoid mixture (α-terpinene: 59.7%; p-cymene: 22.4%; d-limonene: 17.9%), and Repel Aves, based on methyl anthranilate (30%), are products intended for plant protection, although they are not approved for seed treatment. The inclusion of these products in the experiment aimed to evaluate their potential repellent effect against birds, as well as their insecticidal and fungicidal properties. These products were applied at doses of 125, 250, 500, and 1000 ppm (µL active substance per kg of seed). Prior to planting, germination tests (25°C/7 days) (Cojanu et al., 2024) were conducted on seeds treated with the experimental products Requiem Prime and Repel Aves at different concentrations. For the experiment conducted at S.C.D.A. Brăila, two additional products designed to support plant development and improve resistance to biotic and abiotic stress conditions were tested: BioSeed (10 L/t), based on plant extracts, plant and fruit oil, and Biosem (10 L/t), which includes a complex of microelements, plant extracts, neem oil, organic matter, microorganisms, *Trichoderma* fungi, humic acids, and fulvic acids. In the experiment at A.R.D.S. Turda, three replications were established for each treatment, while at A.R.D.S. Brăila, four replications were conducted, with each replication covering an area of 10 m².

Statistical analysis. The data obtained from the evaluation of plant emergence were subjected to statistical analysis using GraphPad Prism software version 7.05. The Shapiro-Wilk test was used to verify the normality of the data distribution, and the Levene test was employed to assess the homogeneity of variances. Subsequently, to highlight significant differences between treatments and evaluation periods, the ANOVA test was applied. For comparison of differences between treatments, the Tukey test ($p < 0.05$) was used to identify groups of treatments that showed significant differences between them. Values are presented as mean (arithmetic average) ± standard deviation (SD) for each treatment variant, evaluation period, and evaluated parameter.

RESULTS AND DISCUSSIONS

At A.R.D.S. Turda, plant emergence was monitored at three distinct stages: 10 days after sowing (14.05.2024), on 24.05.2024, and on 05.06.2024 (Table 1). Statistical analysis revealed significant differences between treatments and between evaluation periods. The ANOVA test indicated a p-value < 0.0001, confirming the existence of statistically significant differences between the mean values of emerged plants across the three evaluated periods.

Table 1. The emergence of plants from treated maize seeds (Mean ± SD)

Variants	14.05.2024 (Mean± SD)	24.05.2024 (Mean ± SD)	05.06.2024 (Mean ± SD)
Control	42.0 ± 0.0	30.3 ± 7.4	28.0 ± 3.6
Requiem Prime 125 ppm	42.0 ± 0.0	27.7 ± 5.1	30.3 ± 2.1
Requiem Prime 250 ppm	42.0 ± 0.0	27.7 ± 4.9	30.7 ± 2.5
Requiem Prime 500 ppm	42.0 ± 0.0	19.7 ± 8.6	25.0 ± 1.7
Requiem Prime 1000 ppm	42.0 ± 0.0	29.3 ± 5.0	28.7 ± 5.8
Repel Aves 125 ppm	42.0 ± 0.0	27.3 ± 6.1	26.0 ± 3.5
Repel Aves 250 ppm	42.0 ± 0.0	32.0 ± 5.1	32.7 ± 2.1
Repel Aves 500 ppm	42.0 ± 0.0	27.3 ± 4.4	27.3 ± 2.3
Repel Aves 1000 ppm	42.0 ± 0.0	**4.3 ± 1.2**	**2.3 ± 1.5**
Raiza mix	42.0 ± 0.0	26.7 ± 6.7	22.7 ± 5.7
Mycoforce	42.0 ± 0.0	28.0 ± 3.6	25.3 ± 3.9
Signal 300 ES	42.0 ± 0.0	29.0 ± 5.0	30.0 ± 3.1

According to the Tukey test, the applied treatments produced varied results. The variant treated with Repel Aves 250 ppm recorded the highest emergence rates in all evaluation stages, with an average of 42.32 ± 5.1 and 32.7 ± 2.1 emerged plants, respectively. In contrast, the variant treated with Repel Aves 1000 ppm showed a significant decrease in the number of emerged plants, with an average of 4.3 ± 1.2 at the evaluation on 24.05.2024 and only 2.3 ± 1.5 at the final evaluation on 05.06.2024.

Treatments with Raiza mix and Requiem Prime 500 ppm also recorded lower emergence rates compared to other variants, suggesting a possible negative influence on seed germination under the specific conditions of 2024.

The multiple comparison test (Tukey) highlighted significant differences between the results obtained at the evaluation period on 14.05.2024 compared to the subsequent periods. The effectiveness of the treatments was significantly higher in the 14.05.2024 evaluation compared to 24.05.2024 ($p = 0.0044$) and 05.06.2024 ($p = 0.0005$).

This indicates an important influence of the evaluation timing on the results, particularly for treatments that showed superior emergence rates in the first evaluation.

Regarding the rotted plants (Table 2), the Repel Aves 1000 ppm variant recorded the highest values, with a very large number of rotted plants in all replications (37-38 plants), indicating a high susceptibility to rotting and a negative effect of this treatment. The Mycoforce variant showed low values for rotted plants, with 0 to 3 plants affected, suggesting a protective effect against rotting. The multiple comparison Tukey test highlighted significant differences between treatments. The variant treated with Repel Aves 1000 ppm recorded significantly more rotted plants compared to the control ($p < 0.0001$) and compared to the treatments with Mycoforce, Raiza mix, Repel Aves 125 ppm, and Repel Aves 250 ppm ($p < 0.0001$). This result indicates that treatments with Repel Aves 125 ppm and Repel Aves 250 ppm demonstrated superior efficiency in preventing plant rotting.

Regarding the wireworm (*Agriotes* sp.) attacks (Table 2), the infestation was minor, with most variants recording a low number of affected plants (between 1 and 5). However, the variant treated with Repel Aves 250 ppm showed the best effect compared to the other variants, followed by the treatment with the chemical product Signal.

The crow attacks (Table 2) in the Repel Aves 250 ppm variant appeared to be the lowest, indicating that the product is effective in preventing crow attacks, fulfilling its primary purpose and contributing to the success of the crop. Alternative treatments that demonstrated promising effects in this regard include Requiem Prime at 125 ppm and 250 ppm.

The lowest values of cutworm (*Agrotis segetum*) attacks were recorded in the variant treated with Requiem Prime 250 ppm, followed by Repel Aves 250 ppm. Raiza mix and Mycoforce recorded slightly higher values, with up to 5 plants attacked by cutworms, indicating a lower level of protection against this pest.

Table 2. The influence of maize seed treatment (Turda 344 hybrid) on the number of plants attacked by diseases and pests

Variants	No. of rotten plants (Mean ± SD)	No. of plants attacked by wireworms (*Agriotes* sp.) (Mean ± SD)	No. of plants attacked by crows (Mean ± SD)	No. of plants attacked by turnip moth (*Agrotis segetum*) (Mean ± SD)
Control	5.0 ± 1.0	1.67 ± 0.58	6.0 ± 2.65	1.33 ± 1.53
Requiem Prime 125 ppm	3.0 ± 1.0	2.0 ± 1.0	4.33 ± 0.58	2.33 ± 0.58
Requiem Prime 250 ppm	3.0 ± 1.0	2.33 ± 0.58	4.33 ± 1.15	1.67 ± 1.53
Requiem Prime 500 ppm	5.0 ± 1.0	3.67 ± 0.58	6.0 ± 1.0	2.33 ± 0.58
Requiem Prime 1000 ppm	3.67 ± 3.06	2.33 ± 1.53	5.0 ± 1.0	2.33 ± 1.15
Repel Aves 125 ppm	4.33 ± 0.58	3.0 ± 1.0	5.33 ± 3.06	3.67 ± 1.53
Repel Aves 250 ppm	4.33 ± 3.21	1.67 ± 1.53	2.33 ± 1.15	2.33 ± 1.53
Repel Aves 500 ppm	4.0 ± 1.0	3.33 ± 1.53	7.67 ± 1.15	2.33 ± 1.15
Repel Aves 1000 ppm	37.33 ± 0.58	1.33 ± 0.58	0.33 ± 0.58	0.67 ± 1.15
Raiza mix	2.67 ± 0.58	3.0 ± 2.65	10.33 ± 3.06	3.33 ± 1.53
Mycoforce	1.33 ± 1.53	3.67 ± 1.53	8.33 ± 2.52	3.33 ± 3.06
Signal 300 ES	3.33 ± 2.08	1.67 ± 1.15	4.67 ± 1.53	2.33 ± 2.08

Regarding maize production, the One-way ANOVA test indicated the presence of significant differences between treatments ($p < 0.0001$). The Tukey multiple comparison

test confirmed that the treatment with Repel Aves 1000 ppm recorded a significantly lower yield compared to the control ($p = 0.0094$) and the treatments with Mycoforce, Raiza mix, Repel Aves 125 ppm, and Repel Aves 250 ppm ($p < 0.001$). This result suggests a potential incompatibility of this treatment with the Turda 344 hybrid.

The treatments with Repel Aves 125 ppm and Mycoforce also recorded high average yields, indicating a positive effect on production. The Raiza mix treatment showed good values, although slightly lower than the most effective variants, suggesting a moderate yet consistent efficacy.

These results indicate that the choice of seed treatment can significantly influence the yield of the Turda 344 maize hybrid, with the Repel Aves 250 ppm variant appearing to be the most suitable option for optimizing the yield of this hybrid (Table 3).

Table 3. Production results obtained from the experience with maize seed treatments (Turda 344 hybrid)

Crt. nb.	Variants	Production (kg/ha) (Mean ± SD)
1	Control	3858.67 ± 130.86
2	Requiem Prime 125 ppm	4486.0 ± 536.61
3	Requiem Prime 250 ppm	4671.67 ± 893.29
4	Requiem Prime 500 ppm	4766.67 ± 1334.67
5	Requiem Prime 1000 ppm	4126.0 ± 875.65
6	Repel Aves 125 ppm	5336.33 ± 577.62
7	Repel Aves 250 ppm	5798.33 ± 547.26
8	Repel Aves 500 ppm	4822.67 ± 742.03
9	Repel Aves 1000 ppm	1067.0 ± 502.33
10	Raiza mix	5263.0 ± 536.54
11	Mycoforce	4764.33 ± 710.02
12	Signal	4007.67 ± 1249.23

At A.R.D.S. Brăila, the application of different treatments to maize seeds (Harmonium hybrid) resulted in significant differences for certain evaluated parameters, while for others, the treatment effects showed no statistically significant variations.

The number of cobs per 10 m² showed similar values across all treatment variants ($p > 0.05$), except for the treatments with Repel Aves 1000 ppm, which differed significantly ($p = 0.03$) from the Raiza mix treatment. Lower values recorded for Biosem (39 ± 5.57) and Raiza (36 ± 4.0) indicate a lower efficacy of these treatments in this regard (Table 4).

Regarding harvest moisture, the differences between treatments were also significant ($p < 0.01$), with the lowest values observed in the treatments with Bioseed and Raiza, confirming the positive impact of these products on reducing harvest moisture. The lowest moisture values were recorded for Bioseed (14.47 ± 0.35), Raiza (14.67 ± 0.47), and Requiem Prime 125 ppm (15.78 ± 0.64), which is favorable for improving storage efficiency and reducing drying costs. Conversely, the higher values recorded for Requiem Prime and Repel Aves (up to 20.75%) may involve additional costs for moisture management.

In contrast, for the parameters hectoliter mass (HM), thousand kernel weight (TGW), and grain yield (%), the statistical analysis results showed no significant differences between treatments ($p > 0.05$). The hectoliter mass values indicated superior grain quality in the variants treated with BioSeed (73 ± 1.13) and Signal (73.17 ± 0.55), suggesting good dry matter accumulation and superior grain quality. The highest TGW was recorded in the variant treated with Bioseed (327.36 ± 7.47), followed by Raiza and Signal (approximately 320 g) (Table 4), indicating optimal grain development. Lower values observed in the treatments with Requiem Prime (approximately 298-303 g) suggest reduced efficacy of these products in enhancing grain size.

Regarding grain yield, the treatments with BioSeed (89.02 ± 0.83) and Biosem (89 ± 0.21) showed the best results (Table 4), demonstrating the strong efficiency of these products in improving productivity. In comparison, the

control variant had a lower yield (88.04 ± 0.73), indicating that most treatments contributed to improving the final yield.

The average maize yields, depending on the applied treatment, ranged from 5,445 kg/ha for the untreated control to 8,848 kg/ha for the Repel Aves 125 ppm treatment. The highest yield levels were recorded for Repel Aves 125 ppm (8,848 kg/ha), Repel Aves 250 ppm (8,423 kg/ha), and Requiem Prime 1000 ppm (8,348 kg/ha). In contrast, the control exhibited the lowest average production. Although statistical tests (ANOVA and Tukey HSD) did not reveal significant differences between treatments, some variants stood out due to high production values combined with low variability among replicates, which gives them practical relevance. In particular, treatments based on Repel Aves 125 ppm and 250 ppm demonstrated an optimal balance between productivity and consistency, suggesting that these products may represent promising technological solutions for increasing maize yield.

The results obtained in this study are supported by previous research that examined the efficacy of methyl anthranilate as a bird repellent and seed treatment for protecting crops. Various studies have highlighted the potential of this compound in preventing bird attacks and improving seed emergence and plant development.

A study conducted by Dolbeer et al. (1991) demonstrated that methyl anthranilate is an effective bird repellent, significantly reducing seed consumption when treated with this compound. Tests performed in controlled environments indicated that birds actively avoided treated seeds, confirming the aversive effect of the substance. Furthermore, research conducted by Avery et al. (1998) emphasized that methyl anthranilate is a promising compound for protecting seeds from birds, particularly in maize and rice crops. The authors noted that this compound acts by triggering an unpleasant sensory reaction, causing birds to avoid treated seeds.

A study by Lacy et al. (2018) compared the efficacy of three chemical substances in protecting maize crops from bird damage. The results showed that methyl anthranilate was among the most effective treatments tested, significantly reducing seed consumption and consequently limiting economic losses. Additionally, Barzen and Ballinger (2018) analysed the application of methyl anthranilate for protecting maize and sunflower crops. In this context, treatments based on methyl anthranilate proved effective in reducing bird attacks, particularly in the early stages of crop development.

Although positive results were evident in numerous experiments, research conducted by Kaiser et al. (2021) indicated that the efficacy of methyl anthranilate decreases under improper application conditions. For instance, in sunflower crops, treatments applied directly to mature plants did not achieve the expected efficacy, as the substance did not sufficiently penetrate the vegetative structures, reducing effective contact with birds.

In this study, the application of Repel Aves 250 ppm, a product based on methyl anthranilate, demonstrated significant efficacy in protecting maize crops from bird attacks and pests. The results align with previously published data, confirming that applying this substance at moderate concentrations (250 ppm) provides effective protection without generating phytotoxic effects.

A study conducted by Drăghici et al. (2012) highlighted the positive effects of the BioSeed 3+ biostimulant on the germination of *Capsicum annuum* seeds. In this study, the application of BioSeed 3+ at the optimal concentration (C1) resulted in a 150% increase in the germination rate three days after sowing and a 92% increase after 10 days, compared to the control group. This effect was attributed to the product's ability to stimulate the rapid formation and development of the root system, contributing to vigorous seedlings. Additionally, the study emphasized that BioSeed significantly reduced the germination period, which has important economic implications by lowering energy costs for greenhouse heating.

Correlating these observations with the results of our experiment, it is evident that the BioSeed product, applied to maize seeds under the challenging climatic conditions of the 2024 agricultural year, also achieved positive results, contributing to improved plant vigor and higher yields.

Studies conducted on crops such as barley and beans highlight different behaviors of the

Biosem product, underscoring the importance of biological and agroclimatic factors in its efficacy. In barley crops, Biosem demonstrated high efficiency in combating foliar diseases when applied to seeds (Popescu and Ivan, 2024), while in bean crops, Biosem had limited efficacy against fungal diseases but showed beneficial effects in supporting plant vigor during the early growth stages (Podea et al., 2024).

Table 4. Production results for maize crop, in order to determine the quality indices under the conditions of applying seed treatments (Harmonium hybrid)

Variants_	Number of cobs /10 m^2 (Mean ± SD)	U%_ (Mean ± SD)	HM (Mean ± SD)	TKW (Mean ± SD)	Grain yeld (%) (Mean ± SD)	Production (kg/ha) (Mean ± SD)
Control	44.5 ± 5.07	17.12 ± 1.82	72.4 ± 1.37	288.09 ± 34.34	88.04 ± 0.73	5445±864
Repel Aves 125 ppm	65.0 ± 6.38	17.18 ± 1.64	72.15 ± 0.88	307.01 ± 23.95	88.69 ± 0.29	8848±720
Repel Aves 250 ppm	60.67 ± 10.69	19.5 ± 2.15	71.37 ± 1.8	328.53 ± 37.94	88.38 ± 0.87	8423±611
Repel Aves 500 ppm	51.25 ± 18.77	18.77 ± 2.17	71.03 ± 2.24	345.57 ± 45.34	88.13 ± 1.61	7685±2440
Repel Aves 1000 ppm	67.0 ± 4.24	18.38 ± 1.22	72.0 ± 0.45	315.21 ± 31.41	84.8 ± 6.67	7763±2962
Requiem Prime 125 ppm	62.75 ± 7.23	15.78 ± 0.64	72.5 ± 0.79	279.4 ± 25.37	84.2 ± 7.15	7350±2152
Requiem Prime 250 ppm	62.5 ± 4.95	20.75 ± 0.78	70.15 ± 1.77	321.11 ± 39.44	86.66 ± 1.34	6930±2046
Requiem Prime 500 ppm	45.5 ± 22.22	16.95 ± 1.77	71.78 ± 0.62	286.14 ± 14.38	88.6 ± 0.47	6395 ± 2975
Requiem Prime 1000 ppm	62.75 ± 9.6	16.82 ± 0.95	72.3 ± 0.86	303.97 ± 32.47	87.4 ± 3.67	8348±2089
Bioseed	52.0 ± 4.58	14.47 ± 0.35	73.0 ± 1.13	327.36 ± 7.47	89.02 ± 0.83	6870±725
Biosem	39.0 ± 5.57	15.0 ± 0.96	72.5 ± 0.44	323.11 ± 12.11	89.0 ± 0.21	5807±501
Raiza mix	36.0 ± 4.0	14.67 ± 0.47	71.77 ± 0.31	322.25 ± 31.7	88.82 ± 0.27	6033±591
Signal	39.67 ± 14.43	15.27 ± 0.92	73.17 ± 0.55	320.78 ± 8.77	88.56 ± 0.69	6493±2473

CONCLUSIONS

Based on the results obtained from the experiments conducted at A.R.D.S. Turda and A.R.D.S. Brăila, significant differences were observed between the treatments applied to maize seeds. The efficiency of the treatments varied depending on the location and hybrid, emphasizing the importance of adapting plant protection products to specific agroclimatic conditions and the genetic characteristics of each hybrid.

The product Repel Aves, based on methyl anthranilate (30%), demonstrated its effectiveness in protecting maize crops against crow attacks, confirming the primary purpose of the treatment - its repellent effect. This result is particularly valuable in regions where birds can cause significant losses in the early stages of vegetation. In addition, the application of Repel Aves at a concentration of 250 ppm contributed to uniform and vigorous plant emergence, suggesting a possible indirect beneficial effect on seed vigor or reduced losses in the early growth phase.

In contrast, treatments with Repel Aves (1000 ppm) showed a pronounced negative effect, with inferior results across most evaluated parameters, suggesting potential phytotoxicity or incompatibility, especially with the Turda 344 hybrid.

Treatments with Mycoforce and Raiza mix demonstrated moderate beneficial effects, showing increased efficiency in reducing plant rotting and contributing to achieving competitive yields.

The results confirm that biological treatments applied to maize seeds can serve as effective and sustainable alternatives for crop protection, promoting higher yields and reducing the negative environmental impact. Notably, the Repel Aves 250 ppm concentration proved to be the most promising option for optimizing production and protecting against pests while offering an eco-friendly solution for modern agriculture.

However, given the variability of environmental factors and potential differences between hybrids, further testing on larger areas and under diverse agroclimatic conditions is necessary to

confirm the stability and efficiency of these products across different cropping scenarios.

ACKNOWLEDGEMENTS

This research was supported by the Ministry of Agriculture and Rural Development, Romania, through the ADER Program 2022-2026 within the ADER 2.1.3 project "Testing and analyzing the technical and economic efficiency of using eco-friendly/biological insecto fungicide products for the treatment of maize, sunflower, wheat/fall cereals, and rapeseed seeds, as an alternative to conventional products".

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

ON THE GROWTH AND DEVELOPMENT PROCESS OF GRASS MIXTURES AND LEGUMES FOR RIPARIAN GRASSLAND RENOVATION

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Abstract

The availability of solar radiation is a key environmental factor in the development of plant species, the intensity and regime of light being important for plant morphology and production, directly influencing the dominant species during a certain period of a season. To study the development of vegetation in riparian grasslands, 36 experimental plots were established. The experiment was located in Mogoșești village, Dragomirești commune, Dâmbovița county, approximately 1 km from the Dâmbovița river, where the environmental conditions are similar to the areas occupied with grasslands near the Dâmbovița River. Two variants were considered: fertilized and unfertilized. The plots were sown with various varieties, combined, of Medicago Sativa, Lotus Corniculatus, Lolium, Festuca, Poa, etc. Vegetative growth was good, including on some plots in the unfertilized lot; however, in the absence of fertilization and deficiencies in the microelements necessary for plant development, the vegetation in the unfertilized plots showed lower LAI values, and lower density, and lower quantities of dry matter.

Key words*: leaf area, quantitative analysis, crop growth, development stages, fertilization.*

INTRODUCTION

In the field of grassland sciences, to address the new challenges arising from climate change and increasingly significant human disturbances, an increasing number of studies have been conducted on topics such as biodiversity conservation, grassland restoration, as well as the sustainable use and management of grassland resources and the enhancement of their multifunctional role (Bărbulescu et al., 1991; Culicov et al., 2024; Wang et al., 2025). In his long-term study (from 1900-), Zhao (2023) showed that ecology and environment-related topics such as biodiversity conservation, land use and soil erosion, climate change, and paleoenvironment have developed rapidly. The increase in the ranking of biodiversity conservation during the study period was the fastest. The ranking of technology-related topics such as remote sensing and numerical modeling has also increased in recent decades. The place of traditional themes (e.g., biological nitrogen fixation, grazing, plant nutrition, germplasm and reproduction, fodder cultivation, and animal production) has recorded a sharp decrease. The assessment of the resilience of meadows and pastures depends on a good understanding of the interactions of environmental factors, the type and properties of vegetation, and anthropogenic activity (Merengo et al., 2025). Vegetation has spontaneously settled on the vast majority of riparian grasslands without direct human intervention, and consequently, it has a diverse characteristic also due to the dynamic nature of ecopedoclimatic conditions. It presents seasonal fluctuations in terms of floristic composition, which generates varied aspects during the vegetation season, depending on the phenology of the component species (Dunea et al., 2021; Vîntu et al., 2024). The bibliographic study using the resources of the Clarivate Web of Science database (bibliographic analysis carried out in April 2023) showed that the field of study of riparian grasslands is increasingly being approached. Using the syntax "*riparian grassland*" in search, yielded 1231 articles, of which 4 are in the highly cited category.

Generally, these articles mainly refer to the interaction of land use/cover (LULC) with water pollution levels or other environmental processes. For a more specific search, the term riparian grassland canopy was used, yielding 91 articles (88 articles, 8 proceedings, 1 chapter, and 1 review article). Finally, the phrase that generally characterizes the objectives of the present study was used, namely "*riparian grassland canopy growth and development*". The search results summarized in a single article that referred to a poplar plantation regarding the management of growth factors. For this reason, the addressed topic regarding the study of the agronomic potential of riparian grasslands presents perspectives in terms of the originality of the subject and the involvement of modern technologies for monitoring and modeling the growth and development of the specific vegetation cover of these important agro-ecosystems for biodiversity conservation, carbon storage and socioeconomic support of rural communities. Riparian grasslands are a special type of grassland that exists in the transition zone between terrestrial (land-based) ecosystems and aquatic (water-based) ecosystems like rivers, streams, lakes, and wetlands. These areas have a unique combination of characteristics due to their proximity to surface waters, making them distinct from the drier upland grasslands. Key characteristics of riparian grasslands can be summarized as: a higher soil moisture content compared to adjacent uplands, influenced by the nearby water body and potentially by seasonal flooding or a higher water table, a potentially higher fertility of the soils in riparian areas - they are often alluvial, being formed from sediments deposited by the adjacent water (such soils can be very fertile and may have different textures and drainage properties compared to upland soils), a greater diversity of plant species than upland grasslands (existence of a mix of grasses adapted to moist conditions, sedges, bullrushes, and various herbaceous plants from other botanical species; in some cases, they might even have scattered shrubs or trees, blurring the line with riparian woodlands or shrublands), a denser vegetation (typically more lush and dense due to the increased water availability), a proper microclimate (riparian areas tend to be cooler and shadier than adjacent uplands due to the proximity to water and the often denser vegetation; this creates a unique microclimate that influences the types of plants and animals that can thrive there).

The riparian grasslands significantly contribute to biodiversity conservation by offering a habitat for various plant and animal species, while also impacting soil stability and the hydrological cycle (Dincă and Dunea, 2018; Yang et al., 2019). These grasslands are vital in protecting aquatic ecosystems, as they act as buffers against eutrophication caused by agricultural activities. By filtering and reducing pollutants like phosphorus and nitrogen, riparian grasslands help prevent water quality degradation having the potential to safeguard the aquatic environments (Dunea et al., 2019; Dunea et al., 2021). Additionally, riparian grasslands are important for carbon storage, aiding in climate change mitigation and creating a favorable microclimate for nearby areas (Wang et al., 2025).

In this complex context, the current study examined the growth and development of various grasses and legumes suitable for riparian grasslands under fertilization while all plots received equal conditions in terms of humidity. The research assesses the impact of these plant species on biodiversity, biomass productivity, and soil stability, considering their effects on water retention and nutrient use efficiency. The study also explores the adaptability of the tested species to the dynamic conditions of riparian microclimatic conditions and their ecological interactions, aiming to identify the optimal combinations for improving fodder quality and maintaining long-term soil fertility (Musat et al., 2023). In a previous study (Dincă et al., 2023), we presented the forage yield and chemical composition of the material collected from the tested variants, both in unfertilized and fertilized conditions.

These approaches promote sustainable development and ensure the productivity of riparian grasslands with minimal human intervention.

MATERIALS AND METHODS

Study Site Description and Experimental Design

The current results were obtained from field experiments performed on plots near Dambovita

River riparian area in Dragomiresti village (Lat. N44°53'7.40", Long. E 25°24'6.89"). The full description of the methodology including field preparations and operations, and plots monitoring were presented in Dincă et al. (2023). A total of 36 experimental plots was arranged in 6 randomized blocks, 3 fertilized and 3 unfertilized with 3 repetitions (6 × 2 × 3) (Table 1 and Figure 1).

Table 1 Species used for field experimental trials

Species	***Percentage (%)***
V1	
Medicago sativa -Valahia variety	100%
V2	
Medicago sativa - Pomposa variety	100%
V3	
Lotus corniculatus - Leo variety	100%
V4	
Lolium perenne 1	45%
Festuca rubra	25%
Lolium perenne 2	30%
V5	
Festuca arundinacea - Variety 1	37%
Poa pratensis	9%
Lolium perenne	9%
Festuca arundinacea - Variety 2	37%
Festuca arundinacea - Variety 3	8%
V6	
Festuca rubra - Adio	10%
Lolium perenne - Solen	25%
Lolium perenne - Nira	10%
Lolium perenne - Pinia	5%
Poa pratensis - Appalachian	8%
Festuca arundinacea - Rendition	3.90%
Festuca arundinacea - Kora	1.10%
Lolium multiflorum - Kajana	4.60%
Lolium multiflorum - Mowestra	32.40%

For the monitoring of environmental factors, soil moisture, and rainfall regime, the automatic DeltaLink GP2 data acquisition (Delta-T Devices, Cambridge, UK) system was used. It was equipped with soil moisture sensors and a rain gauge for precipitation measurements (Figure 2).

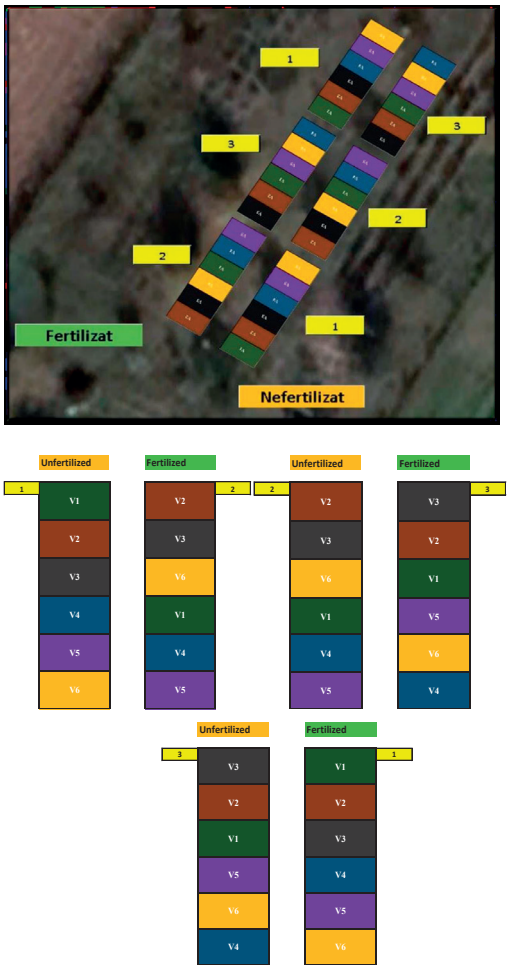


Figure 1. The spatial arrangement of the experiment



Figure 2. Soil moisture sensor and rain gauge

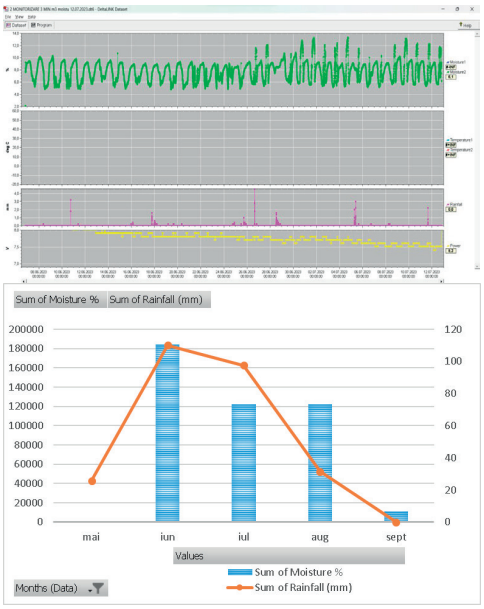


Figure 3. DeltaLINK 3.9 Data acquisition interface and the results of field microclimate monitoring (soil moisture and precipitations)



Figure 4. Height measurements of the uniform and mixed canopies and biomass sampling

Figure 3 presents the soil moisture and precipitation regime during the vegetative growth period.

Samplings of biomass and height measurements were performed several times during the vegetation season, respectively at each cutting cycle (Figure 4).

Leaf area index was measured with a Delta-T Devices SS1 SunScan System that included the SunScan Probe and the BF5 Reference PAR Sensor that was placed outside the canopies. The probe has 64 PAR (Photosynthetically Active Radiation) sensors, connected via RS-232 cable to a Trimble PDA device (https://delta-t.co.uk/product/sunscan/).

The system measures photosynthetically active solar radiation, automatically calculating the LAI based on the PAR fluxes ratio and providing precise information about how vegetation intercepts direct and diffuse light (Figure 5).

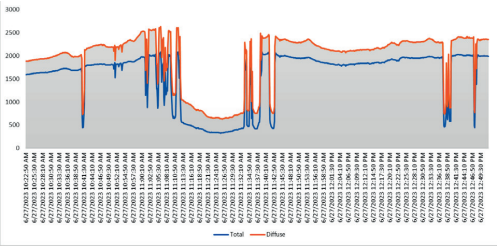


Figure 5. PAR total and PAR diffuse amounts (µmol m^{-2} s^{-1}) recorded during the sampling performed in 27.06.2023 in the experimental field

In this paper, we present the results obtained at the first cutting cycle performed on 27 June 2023, in the first year of cropping.

The microclimate conditions recorded at the time of measurements were as follows:

- PAR radiation: 1060 µmol/m²·s, 891 W/m²;
- Wind speed: 2.7 m/s;
- Atmospheric temperature: 34.2°C;
- Atmospheric humidity: 49%;
- Soil temperature: 24°C.

RESULTS AND DISCUSSIONS

The application of one level of fertilization (120 g m^{-2}) in each variant i.e., 16% (N) total nitrogen, 4.7% (N) nitric nitrogen, 11.3% (N) ammoniacal nitrogen, 5.2% (P_2O_5) phosphorus pentaoxide soluble in neutral ammonium citrate solution and water, 3.4% (P_2O_5) soluble phosphorus pentaoxide, 5.2% (K_2O) soluble potassium oxide, 3% (MgO) total magnesium oxide, 26% (SO_3) total sulfur trioxide, 4% (Fe)

total iron provided the increasing of the measured canopy indicators mostly in each tested variant showing benefits to the growth and development of the canopies.

Canopy height

Clear differences were noted between the fertilized and unfertilized variants, with the fertilized ones having higher heights of the canopy in each variant (Figure 6).

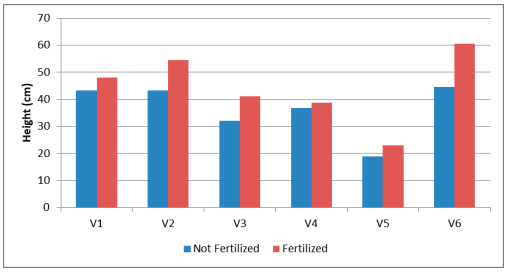


Figure 6. Averaged height measurements (in cm) of the uniform and mixed canopies in the not-fertilized and fertilized variants at the first cutting cycle

Dry matter accumulation

The accumulated dry matter was significantly higher in the fertilized variants compared to the non-fertilized ones, highlighting the benefits of fertilization, except in *Lotus corniculatus* (V3), which showed a decrease (Figure 7).

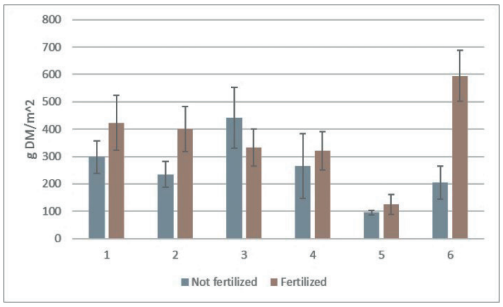


Figure 7. Averaged Dry Matter (DM) accumulation in each tested variant (g DM m^{-2})

Leaf area index

The LAI index indicated that the fertilized variants had a greater ability to intercept light and consequently an increased photosynthetic efficiency. LAI is a vital metric of the growth and development of plant species because it reflects several important aspects of a plant canopy. Therefore, the more leaves a plant has, the higher its LAI, and vice versa. This directly affects the plant's ability to photosynthesize, as leaves are the primary sites where sunlight is captured and converted into energy (Figure 8).

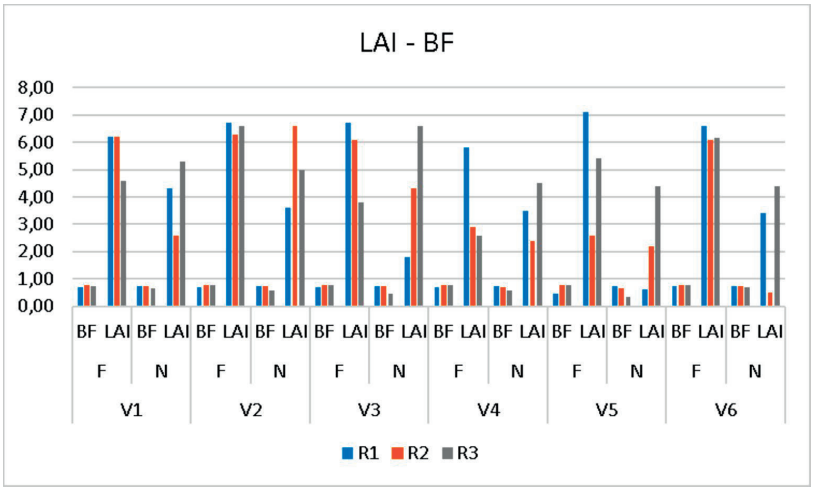


Figure 8. Comparative graph of the LAI (Leaf Area Index) and BF (Beam Fraction) values for the unfertilized (N) and fertilized (F) variants – V1-V6 = number of variant

On the other hand, a high LAI indicates a dense canopy with many overlapping leaves. Conversely, a low LAI suggests a sparse canopy with more gaps between leaves. Canopy density influences how much sunlight reaches the ground and how much water is intercepted by the leaves (Filip et al., 2024). Overall, the best results in terms of the canopy benefits were

obtained by V1, V2 (alfalfa varieties), and V6 (complex grass mixtures) variants, especially in fertilized conditions.

CONCLUSIONS

By measuring LAI, researchers and growers can gain valuable insights into plant health, growth, and ecosystem functioning. The presented results demonstrate that fertilization significantly influences the development and productivity of grass and legume mixtures, except the bird's-foot trefoil. The data obtained indicate that the application of an optimal fertilization regime significantly improves plant height, biomass, and leaf area index (LAI), fundamental aspects for maintaining a productive and balanced ecosystem. These findings are also supported by the literature (Monteith and Unsworth, 2013), which emphasizes the importance of a high LAI in intercepting active photosynthetic radiation and increasing dry matter yield.

The study confirms the clear benefits of fertilization on vegetation development in riparian grasslands, highlighting both ecological and economic advantages. Increasing plant density contributes to reducing soil erosion, improving water retention capacity, and stimulating beneficial interactions between grass and legume species. These results will provide essential data for the development of effective management strategies, contributing to the formulation of sustainable practices for farmers, authorities, and specialists in the field of environmental protection and regenerative agriculture. Further work will consider the utilization of a mixture between V1, V2, and V6 in different proportions of participation in real riparian grassland conditions.

ACKNOWLEDGEMENTS

The research leading to these results received support for publishing from the CNFIS-FDI-2025-F-0421 project financed by the Romanian Ministry of Education and implemented by Valahia University of Targoviste.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

THE WESTERN CORN ROOTWORM (*Diabrotica virgifera virgifera* Le Conte) POPULATION IS INCREASING IN THE SOUTHEAST OF ROMANIA

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Abstract

The western corn rootworm (Diabrotica virgifera virgifera Le Conte) was first detected in Romania in 1996 the western part of the country. Since then, the insects have spread to the east. This paper presents the results of this pest population monitoring in southeast Romania at the National Agricultural Research and Development Institute, Fundulea, Călărași County, maize field. The first detection of this pest in the pheromone traps at NARDI Fundulea was in 2017. This study has presented the results of western corn rootworm fly monitoring at NARDI Fundulea between 2020 and 2024. It has used pheromone traps, KLPfero+ type, from Csalomon®. The highest population of this pest was recorded in 2020, with a total number of 2192 captures, followed by 2023 (1645 captures), 2022 (1521 captures), and 2021 (1020 captures). In 2020, the flight peak was in the first 20 days of July, while in 2021, it was in the first 20 days of August. In 2023, the flight peak was in the last 10 days of July, while in 2024, it was in the last 20 days of July.

Key words*: maize, pest, rootworm, high pressure.*

INTRODUCTION

Western corn rootworm (WCR) *Diabrotica virgifera virgifera* (Le Conte) is the most dangerous pest of the maize crops in North America (Miller et al., 2005; Gray et al., 2009; Gassmann et al., 2012; Manole, 2017; Bažok et al., 2021). Larva is the most harmful stage of this pest, damaging the root system of the maize plants (Spike & Tollefson, 1991; Levine **&** Oloumi-Sadeghi, 1996; Bărbulescu, 2000; Urías-López et al., 2001; Clark **&** Hibbard, 2004; Toepfer et al., 2008). The same authors mentioned that WCR third instar larvae produced higher damage at maize roots. During the feeding process, WCR larvae produced wounds at the plant roots and can cut off secondary and main roots (Kahler et al., 1985; Sutter et al., 1990; Urías-López et al., 2000; Wesseler & Fall, 2010; Furlan et al., 2022). If maize stability is affected, plants can fall on the ground after higher winds or storms (Meissle et al., 2009; Bazok et al., 2021; Amarghioalei et al., 2022). Attacked plants can partially recover, having the stems as a swan's neck (Antonie & Spânu, 2010; Florian et al., 2013; Tanasković et al., 2017). This is the most typical symptom of the WCR larva attack (Bărbulescu, 2000; Toepfer & Kuhlmann, 2006; Roșca & Istrate, 2009; Manole, 2017; Meinke et al., 2021). Damages produced by WCR in the United States exceed 2 billion dollars per year (Wechsler & Smith, 2018; Darlington et al., 2022). Early reports reference damages exceeding 1 billion dollars (Metcalf, 1986; Dun et al., 2010; Wesseler & Fall, 2010). The research made by WCR biology concluded that the maize monoculture favoured this pest (Bărbulescu, 2000; Gray et al., 2009; Sivčev et al., 2012; Manole, 2017; Bažok et al., 2021). New findings suggested that some larvae can appear over two years (Spence et al., 2009; Meinke et al., 2009). In that case, the system maize-soybean, practiced widely in the US Corn Belt, is ineffective for preventing WCR attack (Bažok et al., 2021; Darlington et al., 2022). Chemical control was the most common method used by the farmers to protect maize plants against WCR (Sutter et al., 1990; Van Rozen et Ester, 2010; Inđić et al., 2014; Meinke et al., 2021; Amarghioalei et al., 2025). Seeds treatment was used on a large scale in North

America and Europe (Bărbulescu, 2000; Wesseler & Fall, 2010; Ferracini et al., 2021). GMO maize is used on a large scale in the USA and Canada (Meissle et al., 2009; Deitloff et al., 2016; Bazok et al., 2021; Darlington et al., 2022). However, in the last two decades, there have been many references from the literature concerning WCR resistance to *Cry* toxins from maize GMO crops (Spencer et al., 2009; Cullen et al., 2013; Jakka et al., 2016; Gassmann et al., 2012; Paddock et al., 2021). At the same time, there are many references concerning WCR resistance to insecticides from different chemical classes (Miller et al., 2009; Souza et al., 2019; Meinke et al., 2021). The increase in the WCR populations and the resistance to insecticides and GMO maize can be a serious challenge for farmers in North America. In Europe, WCR is a relatively new pest, and was detected for the first time in former Yugoslavia, in a maize field near Belgrade airport in 1992 (Sivčev et al., 2002). In 1995, this pest was detected in Hungary and Croatia, in 1996 in Bosnia-Herzegovina, in 1998 in Italy (Venezia), in 2000 in Bulgaria, Slovakia, Albania, and in 2002 in France, near Paris (Manole, 2017; Bazok et al., 2021). From the first detection, in 1992, the WCR area in Europe extended, on average, by 40 km each year, ranging from 1 to 80 km/year, depending on weather conditions and crop structure (Kiss et al., 2005). Some studies revealed that in Europe, multiple WCR introductions were made from the North American continent (Ciosi et al., 2008; Bermond et al., 2012). Highest pest densities were registered in the south-east and the centre of Europe (Meinke et al., 2009; Sivčev et al., 2012; Furlan et al., 2022). In Romania, WCR was first detected in 1996, at Nădlac, in the Arad County, situated in the western part of this country, at 300 m from the border with Hungary (Bărbulescu, 2000). Since its first detection, WCR spread to the east of Romania. In the first decade of the 2000s, this pest was already present in the western half of this country (Antonie & Spânu, 2010; Grozea, 2010). The same authors mentioned that the insects expand to eastern areas from Romania at a slower speed because of the Carpathian Mountains chain. More recent data confirm faster WCR spreading to Romanian eastern and south-eastern areas, in Ilfov, Dâmbovița, Teleorman, Ialomița and Constanța Counties, where maize is cultivated on large areas (Manole et al., 2017). In 2015, WCR was reported for the first time in the Central Moldavia area from eastern Romania (Trotuș et al., 2020). Recent data from the same location reveal high WCR adults' density captured in the traps placed in the maize crops (Amarghioalei et al., 2024). This data reveals that Romania could increase the danger of high WCR pest pressure in the maize crops in the future. In this paper, the authors present the results of the WCR flight monitoring, with pheromone traps in the south-east of Romania.

MATERIALS AND METHODS

The flight of WCR adults was monitored at the experimental field of the Agrotechnics Laboratory, from National Agricultural Research and Development Institute Fundulea, Călărași County, Romania (latitude: 44°46' N; longitude: 26°32' E; alt.: 68 m a.s.l.), between 2020 and 2024. At this experimental site, soil type is chernozem with medium texture, humus content of 2.8-3.2%, pH of 6.4-6.8, nitrogen content of 0,17-0,18 %, potassium content of 135-170 ppm, and phosphorus content of 10-25 ppm. In 2020 and 2021, this study used the F423 maize hybrid; in 2022 and 2023, it used the Iezer hybrid; and in 2024, it used the Felix hybrid. These hybrids were created at NARDI Fundulea and were from the FAO 401-500 group (Horhocea et al., 2019; 2024). In the experimental field, soil works, fertilisation, and herbicide applications followed the technology for maize crops.

- In 2020, the maize was sown on 14 April, plants emerged on 28 April, and the harvest was on 17 September.
- In 2021, the maize was sown on 7 May, plants emerged on 15 May, and the harvest was on 5 October.
- In 2022, the maize was sown on 3 May, plants emerged on 10 May, and the harvest was on 4 October.
- In 2023, the maize was sown on May 5, plants emerged on May 12, and the harvest was on 22 September.
- In 2024, the maize was sown on 14 May, plants emerged on 21 May, and the harvest was on 31 October.

For **monitoring the seasonal fly patterns of the WCR adults in the maize field**, four traps, KLPfero+ type with sexual pheromone (4-Methoxyphenyl)-2-propenal, from Csalomon®, a commercial brand of the Plant Protection Institute, Budapest, Hungary, have been placed. The traps were placed on a square 100 m apart from each other. The minimum distance between traps and crop margins was 20 m. The traps were placed in the maize field from the beginning of May until the first 10 days of October (Figures 1 and 2).



Figure 1. Pheromonal KLPfero+ trap used for monitoring seasonal fly of the WCR at NARDI Fundulea (29.05.2020)

The height of the traps was adjusted according to the maize vegetation stage. The pheromones were replaced after 4 weeks during the summer months and 6 weeks in May, September, and October. The traps were assessed two times per week.



Figure 2. Pheromonal KLPfero+ trap used for monitoring seasonal fly of the WCR at NARDI Fundulea (10.07.2023)

Meteorological data were collected from the Davies automatic weather station in the NARDI Fundulea experimental field, located 1500 m from the experimental site. During this study, the station monitored daily air temperature and rainfall from April to September. Table 1 presents the 50-year average monthly temperature and total rainfall from the field site.

Table 1. Temperatures and rainfalls multiyear average at NARDI Fundulea field site from April to September

Month	Temperature 50 years average (°C)	Rainfalls 50 years average (mm)
April	11.3	45.1
May	17.0	62.5
June	20.8	74.9
July	22.7	71.1
August	22.3	49.7
September	17.5	48.5

The data presented in Figure 3 shows that in the field site, located at NARDI Fundulea, in the southeast of Romania, the average temperature registered in the summer months was higher than the 50-year average in all five years of this study. Temperatures recorded in April exceed the multiyear average in 2020, 2022, and 2024, while temperatures recorded in May were closer to the averages compared with other months.

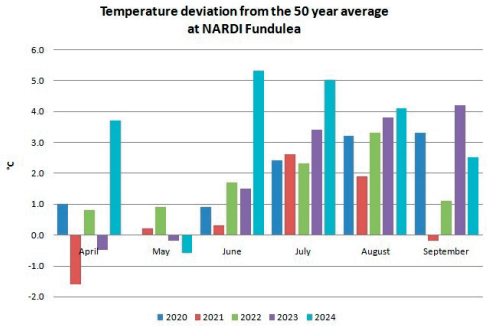


Figure 3. Temperature deviation from the 50-year average at NARDI Fundulea from 2020 to 2024

The highest deviation from the average was in June 2024 (+5.3°C), followed by July 2024 (+5.0°C) and August 2024 (+4.1°C). This year was the warmest in Romania since the meteorological recordings started (Ionita & Nagavciuc, 2025). The lowest deviation from the average was recorded in April 2021 (-1.6°C).

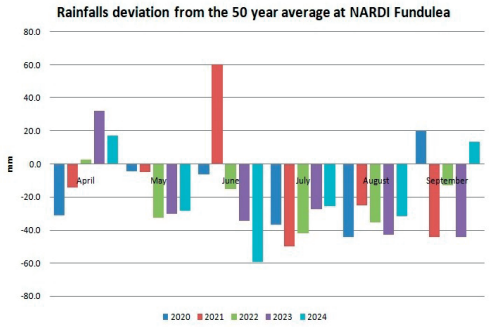


Figure 4. Rainfall deviation from the 50-year average at NARDI Fundulea from 2020 to 2024

Data from Figure 4 reveal that in the field site, located at NARDI Fundulea in southeastern Romania, during the study period (2020-2024), except for June 2021, in all summer months, it has registered lower rainfalls than the 50-year averages. The highest negative deviation from the averages was recorded in July 2024 (-59.3 mm), while the highest positive deviation was in June 2021 (+60.1 mm). Overall weather conditions during this study, in the summer months, were higher temperatures than the averages, and lower rainfall amounts compared with the averages.

Monitoring results are presented as absolute and mean values of the adults WCR captured per trap, the average adults WCR capture per trap in 10 days, and the total number of WCR captured during one maize growing season. During this study, the seasonal fly pattern of this pest at NARDI Fundulea was presented graphically on Microsoft Excel charts.

RESULTS AND DISCUSSIONS

WCR was first detected at NARDI Fundulea, in Călărași County, southeast Romania, on 13 July 2017 (Figure 5), during monitoring by the Research and Development Institute for Plant Protection (RDIPP Bucharest) entomologists in collaboration with NARDI Fundulea. The monitoring used the same traps and pheromones as in this study. The previous year, no WCR adults were observed in the traps (Manole, 2017). No WCR flight was monitored at NARDI Fundulea in 2018 and 2019. Monitoring started again in 2020. Figure 6 shows the total WCR adults captured in the traps during one monitoring season.



Figure 5. WCR adults (*Diabrotica virgifera virgifera*) were first detected at NARDI Fundulea on 13 July 2017

In this study, the highest number of WCR adults captured in the traps was in 2020 (2192 captures), followed by 2023 (1645 captures) and 2022 (1521 captures).

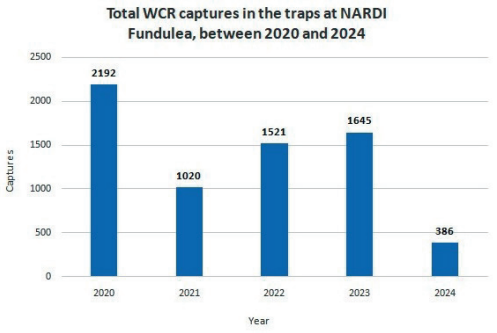


Figure 6. The total WCR captured in the traps at NARDI Fundulea during the maize growing season, between 2020 and 2024

The lowest number of WCR adults captured in the traps during the maize growing season was in 2024. A possible explanation for these high differences between the WCR population in 2024 and the previous year from this study is the unfavorable weather conditions for larva development this year. Drought and high temperature can diminish the number of WCR larvae in the first two instars (Bărbulescu, 2000; Manole, 2017). The same authors mentioned that WCR larva emergence is from the end of May to the end of July, and their life span is, on average, three weeks. Jackson and Elliot (1988) mentioned that at higher temperatures in the soil, where WCR larvae are found, up to 33°C, no larvae in the second instar will survive. Meteorological data

recorded at the field site reveal that in June 2024, the air temperature was the highest deviation from the 50-year average (Figure 3). Between 17 and 19 June 2024, the maximum air temperature exceeded 35°C; on 20 June, it exceeded 38°C (Figure 7).

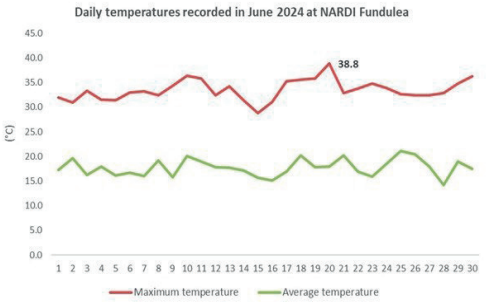


Figure 7. Daily temperatures recorded in June 2024 at NARDI Fundulea

This early heatwave recorded in June 2024 in the southeast of Romania could affect WCR larva development in the soil and decrease the WCR population at the field site where this study was conducted.

Table 2. The WCR flight pattern at NARDI Fundulea, southeastern Romania, from 2020 to 2024

Month	Decade	2020	2021	2022	2023	2024
May	I	0*	0	0	0	0
	II	0	0	0	0	0
	III	0	0	0	0	0
June	I	0	0	0	0	0
	II	0	0	0	0	0
	III	1.75	0	3.25	0	0
July	I	296.75	1.25	10.75	70.50	15.00
	II	141.00	11.50	92.50	127.50	40.25
	III	80.50	43.50	141.75	117.00	26.75
August	I	14.25	68.50	102.25	70.25	20.25
	II	6.25	95.00	25.00	17.50	2.75
	III	4.25	28.50	4.50	8.00	0
September	I	1.00	5.75	0.50	0.50	0
	II	0	0.75	0	0	0
	III	0	0.25	0	0	0
October	I	0	0	0	0	0
	II	0	0	0	0	0
	III	0	0	0	0	0

*the average number of WCR adults/trap in 10 days

Table 2 shows the data concerning the seasonal flight pattern of the WCR adults at the maize field site, NARDI Fundulea, southeastern Romania, between 2020 and 2024.

In 2020, the first WCR adults were captured in the traps on 29 June, followed by a sudden growth of trapped insects. The maximum flight peak was in the first 10 days of July (296.75 captures/trap), when in some traps, there were more than 300 trapped WCR adults (Figure 8), followed by the next 10 days of this month (141.00 captures/trap). In August, fewer WCRs were trapped, while the last captured adults were in the last 10 days of September.



Figure 8. High number of WCR adults trapped in the first 10 days of July 2020, at NARDI Fundulea

In 2021, the first WCR adults were captured on 5 July. Compared with the previous year, the flight curve showed smooth growth. The maximum flight peak was recorded in the first 20 days of August with an average of 68.50 captures/trap in the first 10 days, and 95 adults/trap in the next 10 days, which was the flight peak. The number of trapped WCR adults decreased gradually in the last 10 days of August. However, in the first 10 days of September, it recorded the highest captures from the entire study period. The last WCR adult was trapped on 29 September.

In 2022, the first WCR adults were captured on 27 June. It recorded a few trapped insects in the first 10 days of July. This contrasts with the situation from 2020 when the maximum flight peak was in the first 10 days of July. The flight peak was in the last 10 days of July (141.75 captures/trap). A high trapped WCR adult was in the first 10 days of August. The last insect captured in the trap was on 6 September.

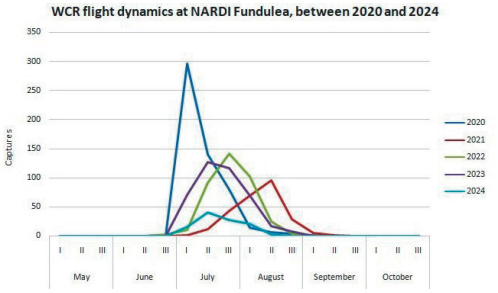


Figure 9. WCR flight dynamics at NARDI Fundulea, between 2020 and 2024

In 2023, the first WCR adults were captured in the traps on 3 July. The maximum flight peak was in the second 10 days of July (127.50 captures/trap), followed by the last 10 days of this month (117.00 captures/trap). In the first 10 days of August, it registered 70.25 captures/trap, followed by a decrease in the flight peak. The last captured WCR adult was on 9 September.

In 2024, the first WCR adults were captured in the traps on 2 July. The data from the first capture was like that of the previous year and 2021. However, the evolution of WCR this year was atypical compared with the previous four years of this study. The flight peak was in the second 10 days of July, but WCR captures in the traps from the maize field site were lower than in the previous years (Figure 9).

After peaking in 2020, the WCR population at the NARDI Fundulea maize field site fluctuated in the following three years and decreased in 2024. More studies are necessary to elucidate the influence of climate change on WCR populations' dynamics.

CONCLUSIONS

This study reveals new information about the evolution of the WCR adults (*Diabrotica virgifera virgifera*) in recent years at NARDI Fundulea, Călărași County, in southeastern Romania, three years after their first detection in 2017.

In 2020, the maize field site at NARDI Fundulea registered the highest number of WCR adults captured in the traps, followed by 2023 and 2022. In 2024, the number of trapped WCR adults was very low. A possible explanation for this is drought and an early heat wave registered in June that can destroy the WCR larvae in the soil.

In 2020, 2022, 2023, and 2024, the WCR flight peak was registered in July, while in 2021, it was in August.

This study demonstrates that there can be a high WCR pressure in the maize fields in southeast Romania, because of which this pest can threaten maize production in the future.

ACKNOWLEDGEMENTS

This research was supported by the National Agricultural Research and Development Institute, Fundulea (Romania).

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

INTEGRATION AND FUNCTIONALITY OF IPM STRATEGIES FOCUSING ON INSECT PESTS IN VARIOUS CROPS

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Abstract

Integrated Pest Management (IPM) is an increasingly used tool for sustainable pest management with the clear aim of reducing adverse environmental impacts and providing long-term economic and ecological benefits. Holistically, this approach aims to manage pest populations through combined and compatible techniques and methods. It is not focused on eradicating and eliminating pesticides but rather on keeping pest populations under control and reducing pesticide use. With this in mind, it is essential to bring to attention of both farmers and researchers in agriculture and plant protection the latest available tools, reflecting the advantages and disadvantages by exemplifying them on crop systems (cereals, vegetables). This review is by far an analysis of what is already used, tested and applicable and on the other hand highlighting the challenges in digital context through the scientific, economic and social potential. All of this helps to make decisions to organize pest management activities and encourage direction towards a safe path for human health and environmental protection by supporting precision agriculture.

Key words*: IPM, insect pests, monitoring, classic and modern tools.*

INTRODUCTION

IPM (Integrated Pest Management) is an approach that integrates information about the life cycles of pests and their interactions with the environment to provide effective control, reducing risks to humans and the environment (Holm, 2022, Zhou et al., 2024).

In order to achieve agricultural sustainability, IPM promotes ecological and sustainable practices, contributing to reducing dependence on pesticides and increasing biodiversity in agricultural systems (Angon et al., 2023; Virteiu et al., 2023).

By combining multiple tactics such as biological control, selective use of insecticides and physical/mechanical methods, it aims to reduce negative environmental impacts and increase efficiency (Riyaz & Kathiravan, 2019).

Although IPM is recognized as an effective strategy, its implementation has been slowed by the complexity of technologies and the lack of financial and human resources (Wearing, 1988; Grozea, 2010). However, it is clear that its implementation has demonstrated a significant decrease in pesticide use and negative environmental impacts, without compromising effectiveness in pest reduction (Moffitt, 1993; Costea et al., 2024).

Another quality is that it supports safe and high-quality food production, contributing to the growing demand for organic food products (Dhawan & Peshin, 2009).

IPM also allows strategies to be adapted according to the specific conditions of the crop and the geographical region, maximizing efficiency and reducing environmental risks (Green et al., 2020).

Another facility is the creation of international research and implementation networks that can help overcome challenges related to communication, knowledge transfer and coordination between countries (Lamichhane et al., 2016).

Considering all these favourable aspects already found at the international and national level, we think it appropriate to detail the information through a review of the sources of IPM application to two important categories of crops (cereals and vegetables) at a global level in diversified systems, for relevant pests and in terms of technological progress, demonstration of efficiency and adoption by farmers.

MATERIALS AND METHODS

Crop Selection

In this review study, we focused on wheat, maize, tomatoes and cucumbers. These are the most commonly grown crops globally, with proven importance and increased research attention.

Wheat is grown globally on an area of approximately 222 million ha, of which just over 2 million are in Romania (Erenstein et al., 2022). According to FAO Stat (2022), maize is grown on approximately 200 million ha globally and 2.5 million ha in Romania(Vili et al., 2022). Tomatoes occupy approximately 5 million ha (open field and greenhouse) being grown with this vegetable, of which in Romania it occupies around 40,000 ha (according to FAO, 2022). Cucumbers are cultivated on an area of 1.2 million hectares, while only at a somewhat unspecified national level, i.e. approximately 5000 ha (FAO Stat, 2022; Anghelache et al., 2023) (Table 1).

Table 1. Summary of statistics on areas occupied by target crops, at global and national level

Crop	Area (ha)		Cultivation System
	Globally	National*	
Wheat	222.000.000	2.000.000	Field
Corn	200.000.000	2.500.000	Field
Tomatoes	5.000.000	40.000	Mixed (field +greenhouses)
Cucumber	1.200.000	5000	Greenhouses and solariums

*Surface area in Romania at the level of 2022

Factors considered

In our searches from various sources at a global level, we took into account the following factors: crop type, cultivation system (organic, conventional), degree of economic development, range of pests, classical methods of keeping them under control and modern methods (as appropriate).

Evaluations and analyses

Based on the information found in the electronic system, methods and the adoption of modern methods by farmers in a non-polluting context. So, mainly we have constructed several diagrams with the most used methods within IPM for each category of crops taken into analysis, as well as the effects on production through pesticide reductions and environmental pollution, for example.

The level of farmers' adoption of modern methods was also assessed through the prism of socio-economic characteristics.

RESULTS AND DISCUSSIONS

It seems that from what was found and analysed, implementing IPM can bring major benefits by reducing pests and increasing yields and farmers' adoption of modern methods in pest IPM.

Pest reduction and yield increase

In wheat crops (Figure 1), the application of IPM (by combining mechanical, biological and chemical methods) was able to reduce aphid populations, mainly with an emphasis on reducing the use of insecticides (even selective) and increasing the share of non-chemical methods. Among the mechanical methods effective in reducing aphid populations (*Schizaphis graminum* Rondani), we highlight sticky traps and yellow liquid traps, and among biological methods, the protection of ladybugs. Clearly, these 2 methods will lead to a reduction in insecticide application. As a result, aphids were reduced, while the ladybug population increased, which is beneficial for the environment and the future. All of this can also lead to yield increases of about 10% of the harvest (Singh & Jasrotia, 2020).

In corn crops, IPM methods to control *Ostrinia nubilalis* (Hubner) (one of the most persistent pests) (Costea et al., 2022) and to increase crop yield can include a combination of biological control (*Trichogramma*, Bt), selective insecticides and crop rotation, according to many studies in this regard (Figure 2). As such, crop rotation reduces the population pressure of the pest and favours natural control by preserving beneficial insects (Camerini et al., 2024).

Biological control with *Trichogramma brassicae* (Bezdenko) has demonstrated similar efficiency to chemical methods, reducing infestation and maintaining mycotoxin levels below EU food safety thresholds, while increasing profitability (Gardner et al., 2011; Razinger et al., 2016). The application of *Bacillus thuringiensis* (Berliner) as a biological

insecticide also provides good control of the pest (Tembo, 2009).

The application of selective insecticides maintains beneficial insect populations and is as effective as conventional chemical methods against *Ostrinia nubilalis* (Vasileiadis et al., 2017).

Genetically modified maize hybrids (Bt maize) provide effective protection against the pest, completely reducing infestation and increasing yield by up to 15% compared to conventional methods (Kocourek & Stará, 2018).

From analysed data for cereals, an IPM strategy cannot be applied only taking into account the crop but also the pest. Practically for each crop and pest an IPM must be developed, depending on the morpho-bio-ecological characteristics.

In tomatoes, IPM for the management of the most common pest (*Tuta absoluta* Meyrick) (Biondi et al., 2018), in field and protected area, combines agronomic, biological, chemical and mechanical methods to reduce the pest population and its impact on tomato crops (Figure 3).

Among agronomic methods, crop rotation and intercropping with repellent plants can reduce the pest population between seasons (Mahmoud et al., 2020). Also, removal and destruction of plant debris contributes to the elimination of larval development sites (Chepchirchir et al., 2021).

Biologically, effective are the use of natural predators (such as *Nesidiocoris tenuis* and *Macrolophus pygmaeus*) that consume the eggs and early larvae of the pest (Giulianotti, 2010) or the application of bioinsecticides with entomopathogenic fungi such as *Beauveria bassiana* and *Metarhizium anisopliae* to infect and destroy the larvae (Buragohain et al., 2021).

The use of attractant-repellent substances to disrupt insect behaviour is also of interest (Desneux et al., 2016).

Sex pheromone traps are widely used to capture males, reduce mating and estimate population density (Guedes & Picanco, 2012).) and light and water traps are used in Nigeria to reduce the number of adults (Oke et al., 2020).

Due to the perishable nature of tomatoes, insecticide use should be minimized and, when necessary, limited to low-toxicity bioinsecticides or selective compounds with short pre-harvest intervals (e.g., *Bacillus thuringiensis*, neem-based formulations).

Integration of these measures helps to maintain long-term effectiveness and prevent insecticide resistance.

In cucumber, IPM for the management of the most widespread pest in both protected areas and in the field, such as *Bemisia tabaci* Gennadius (whiteflies), combines agronomic, cultural, biological, mechanical and chemical methods to effectively reduce pest populations, while reducing the transmission of devastating viruses (Figure 4).

Among the agronomic ones, crop rotation with non-host plants helps to disrupt the whitefly life cycle and reduces infestation pressure. And intercropping cucumbers with repellent plants, such as marigolds or basil, can reduce the attraction of whiteflies (Hegab et al., 2016).

Regarding the cultural ones, the removal of infested plant debris reduces whitefly breeding sites and the use of insect-resistant nets prevents whiteflies from entering greenhouses and open field crops (Stansly et al., 2004).

Biologically, the predatory insect *Phytoseiulus persimilis* and entomopathogenic fungi (*Beauveria bassiana* and *Metarhizium anisopliae*) can control whiteflies populations in greenhouses (Abou-Haidar et al., 2021; Singh & Kaur, 2020).

Mechanically, the use of yellow sticky traps is highly attractive to *Bemisia tabaci*, with capture rates of up to 44% (Hoelmer & Simmons, 2008). In Romania, studies on the effectiveness of traps in pest control are lacking.

Low-toxicity insecticides, such as botanical extracts (neem, aloe), have been shown to be effective against this pest (Darwish et al., 2022).

By implementing these strategies, pest populations can be kept below economic thresholds, reducing the risk of virus transmission and ensuring higher yields.

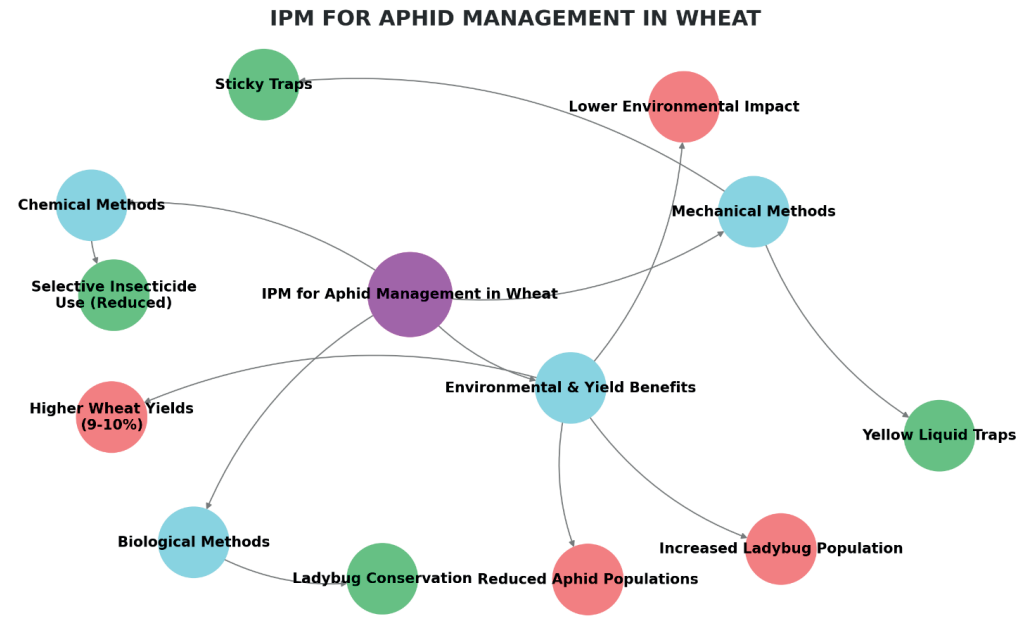


Figure 1. IPM scheme for managing aphids of the genus *Schizaphis graminum* Rondani in wheat crops

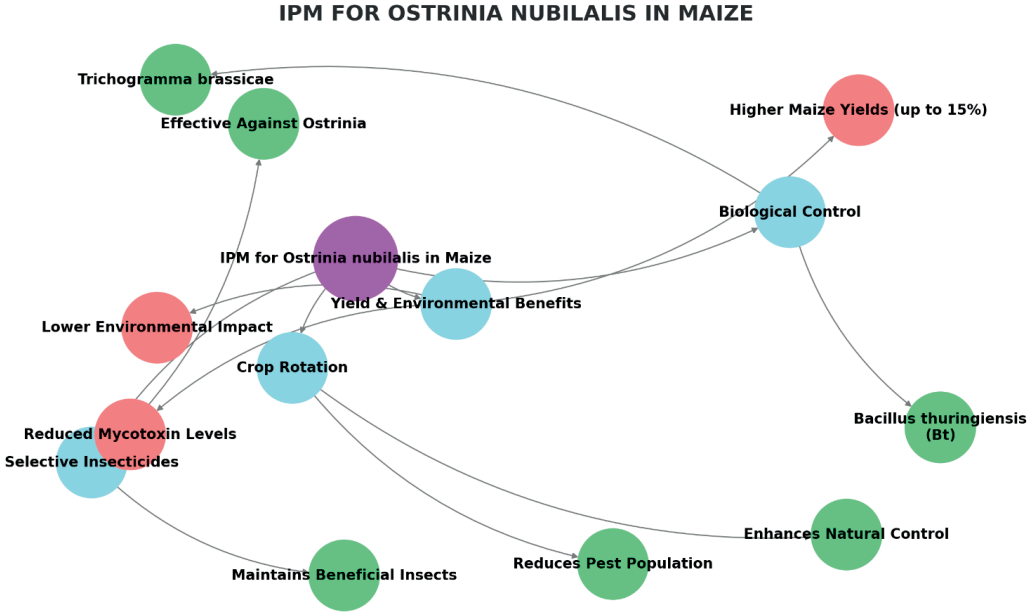


Figure 2. IPM scheme for managing *Ostrinia nubilalis* Hubner in corn crops

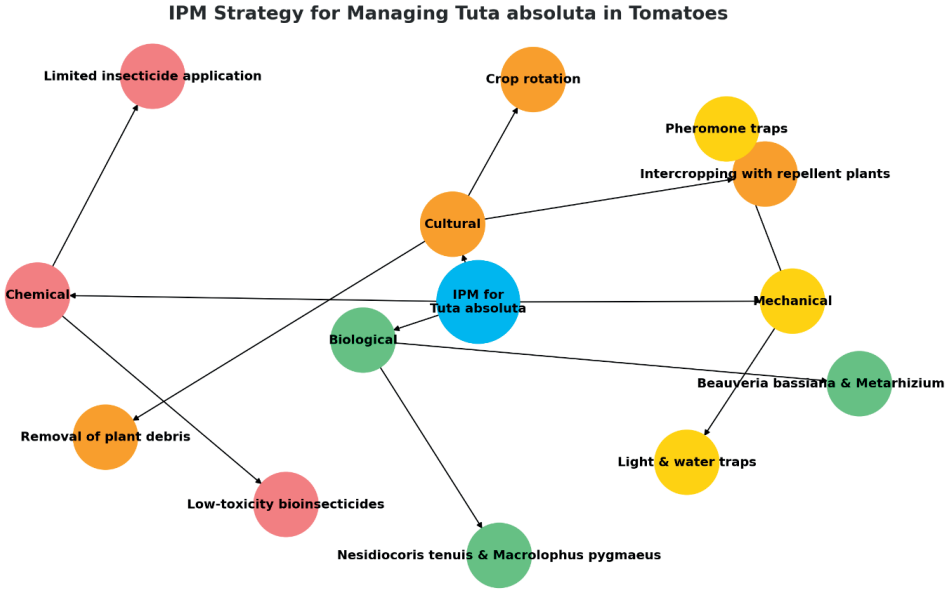


Figure 3. IPM scheme for managing of *Tuta absoluta* Meyrick in tomatoes crops

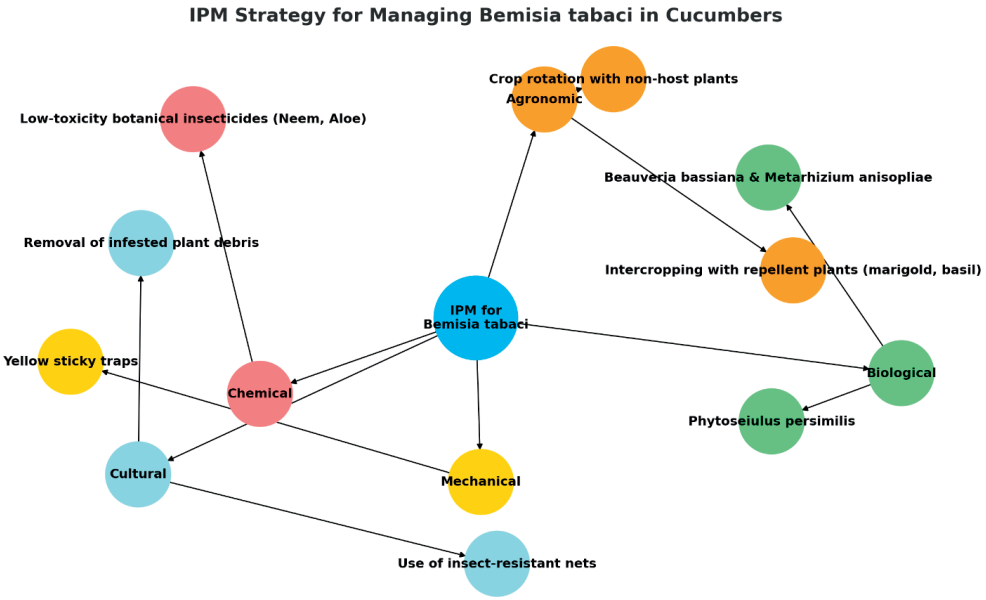


Figure 4. IPM scheme for managing *Bemisia tabaci* Gennadius in cucumbers crops

Farmers' adoption of modern methods in pest IPM

Among the modern methods of managing aphids in wheat crops, unmanned aerial vehicles (UAVs) for pesticide application have been widely adopted by farmers. Their effectiveness has been proven by spraying at low altitude (1.5 m) with optimized droplet distribution, which significantly improves the effectiveness of aphid control (Sun et al., 2022). Digital and smart traps for aphid detection have also been proven effective in estimating aphid populations.

Yellow sticky traps combined with digital monitoring have been proven effective in early detection of aphids in the crop. However, farmers have combined UAVs with classic traps to maintain constant monitoring and reduce the need for chemical control (Jasrotia et al., 2022).
In managing *Ostrinia* populations, new studies have focused on light traps that use UV or fluorescent lamps to attract adults and also on optimizing their energy source for field applications that are easily adopted by farmers. Combining real-time digital monitoring with UAV-based biological control (based on the principle that drones release *Trichogramma* only in infested areas), significantly reduces costs and pesticide use, making the work of farmers much easier (Beres, 2023).
In management of vegetable pests (tomatoes and cucumbers), the GLT trap using yellow light has been shown to be very effective in capturing both whiteflies and *Tuta absoluta* in protected areas. The GLT trap captured three times more insects than conventional yellow sticky traps, making it a promising method that is easy for farmers to apply and maintain non-chemical control (Mirza & Abdi, 2019).
Green LED traps have also been tested to monitor whitefly populations in greenhouses, and have been shown to detect populations earlier than traditional yellow sticky traps, allowing farmers to intervene in a timely manner (Grupe & Meyhöfer, 2024).
UV light traps combined with sex pheromones have been tested to optimize capture efficacy based on the flight activity of *Tuta absoluta* and have proven applicable and effective (Zhang et al., 2023).
AI adoption in agriculture is unevenly distributed, with large commercial farms and younger, more educated farmers leading adoption, while smallholder farmers face financial and infrastructural barriers (Table 2).

Table 2. Economic and social drivers behind farmers' adoption of AI methods

Farm type	AI adoption	Key drivers
Large commercial farms	High	High access to capital, advanced digital literacy
Mid-sized farms	Moderate	Partial access to AI tools, cost-sharing initiatives
Smallholder farms	Low	High costs, lack of access to funding and training
Cooperative farms	High	Knowledge sharing, government incentives

Government policies, digital training programs, and increased access to finance can help bridge this gap (Eunice et al., 2022).

CONCLUSIONS

Based on the findings, it is clear that for the 4 crops taken into account (wheat, corn, tomato and cereals) there are currently IPM approaches that combine classical and modern methods. The terms behind are focused on reducing the use of pesticides and increasing efficiency by increasing production in a context of low environmental impact.
Of course, the acceptance of adopting modern methods is influenced by economic and social terms, the higher the economic level and financial potential and social involvement, the higher the acceptability of the novelty. And the size of the farms is a decisive factor, that is, the larger or more aggregated they are, the higher the acceptability. As a recommendation, combining methods taking into account the crop, pest and socio-economic context is essential in maintaining an IPM at the expected level.

ACKNOWLEDGEMENTS

We would like to thank those who provided us with physical and electronic bibliographic materials upon request and consent to use.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

EFFECTS OF DIFFERENT NITROGEN FERTILIZER RELEASE TYPES AND APPLICATION MOMENTS IN HYBRID SEED MAIZE PRODUCTION

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Abstract

The use of slow-release and controlled-release fertilizers (SRFs and CRFs) is aimed at providing a step towards better N management. Starting from this idea, the aim of the paper is to present the results of the performed research focused on analyzing and comparing the SRFs and CRFs to classical ones, in terms of yield and phenological and phenotypical development of plants in hybrid seed maize production under irrigated conditions, as well as in researching the optimum time of application for each kind of the studied fertilizer. Research has been conducted for 3 years (2020, 2021, and 2022) in irrigated fields located in NE Romania, the experimental factors being the following: Factor A – nitrogen fertilizer product (a1 and a2 = Control 1 and Control 2 – no added nitrogen fertilizer; a3 = ammonium nitrate; a4 = Urea NG; a5 = Multicote (4) 34-0-7); Factor B – fertilizer rate split between seedbed preparation (SbP) and BBCH 16 (b1=100% SbP; b2=50% SbP + 50% BBCH 16; b3=25% SbP + 75% BBCH 16; b4=100% BBCH 16). The obtained results sustain the use of SRFs and CRFs in hybrid seed maize production, which is leading to better yields compared to classical fertilizers, no negative impact on synchronization at pollination time, lower quantities of physical fertilizer product to be used, and reduced number of passes in the field. The best moment of application of nitrogen fertilizers in hybrid seed maize production differs according to fertilizer type: slow-release fertilizers are best applied all at seedbed preparation, controlled-release fertilizers are best applied in 50-50 split between seedbed preparation and BBCH 16 sidedressed, while ammonium nitrate should be applied at BBCH 16 with cultivation.

Key words: *Maize, hybrid seed, slow-release fertilizers, controlled release fertilizers, nitrogen, seed yield.*

INTRODUCTION

Climate change and the continuously and rapidly increase in population are two challenges which are at the forefront of agriculture nowadays and for the foreseeable future. Globally, crop production needs to be increased, upgraded, and safeguarded (Vejan et al., 2021), while reducing the environmental impact and preserving the soil health (Ghumman et al., 2022).

Nowadays, agricultural intensification is the main alternative that encourages farmers to increase agricultural production with limited agricultural land (Kaavessina et al., 2021) and fertilization, as an essential tool of intensification, is a basic technological element of modern agriculture (Șimon et al., 2022). The use of fertilizers is essential for plant growth (Negi et al., 2022), and the effective use of inorganic fertilizers has been responsible for increased food production more than any other input (Penuelas et al., 2023). Fertilizers provide nutrients to plants and they are credited for the improvement in crop yield, which results in exponentially increased fertilizer use worldwide (Lawrencia et al., 2021). Meanwhile, the massive use and misuse of mineral fertilizers resulted in land degradation and impairing the sustainability of many agro-ecosystems (Allam et al., 2021), this having a negative impact on the environment and exacerbating the effects of climate change (Benlamlih et al., 2021; Kaavessina et al., 2021). Thus, the aim of using fertilizers is to reduce the environmental impact and to enhance food quality while maintaining acceptable yields (Spiertz, 2010).

Increase in fertilizer prices coupled with the need to reduce negative ecological effects imposes necessity to have a better correlation between the moment of nutrient consumption and their application especially regarding nitrogen. Among all nutrients, nitrogen is the nutrient most limiting crop production in all areas of the world and is generally applied to soil in the largest quantity (Penuelas et al., 2023; Spiertz, 2010). About 50% of the

increase in food grain production been attributed to fertilizer use and more than 30-40% increase is due to nitrogen fertilizers alone, but about one-fourth to one-third of the applied fertilizer nitrogen is lost as ammonium and nitrous oxide gases to the atmosphere or as nitrates to the surface or ground waters, creating several environmental and health problems (Bahar et al., 2019). Unfortunately, discretionary use of nitrogen fertilizers in the past, in high quantities, have favoured leaching of nitrogen into ground waters and high losses of nitrogen available for plant uptake (Wang et al., 2019; Goulding et al., 2008). Therefore, nitrogen management requires special attention in its use so that the losses can be minimized, and the efficiency maximized (Jat et al., 2012), especially in highly demanding crops where high N rates are used (Arrobas et al., 2022). Practically, it is necessary to optimize N fertilizer use both to meet crop requirements and to reduce N losses (Zheng et al., 2016).

Classical maize fertilization technology has been constructed by practicians around old products of chemical synthesis with 1:1:1 or 1:3:0 N:P:K ratios, urea or ammonium nitrate all which have been developed in the first half of the 20th century (Hergert et al., 2015). The nutrients from these types of fertilizers are assimilated by plants through the concept of solubilization and uptake through the root system, but unfortunately recent research has shown that these classical fertilizers are very sensitive to excess water, with the implied levigation, as well as to volatilization (Schwenke et al., 2022).

New types of fertilizers have been developed in the past decade to better copy the pattern of nitrogen uptake of crops, respectively slow-release fertilizers (SRF) and controlled-release fertilizers (CRF) which use either a polymer coating or nitrification inhibitors to model nitrogen release to follow more accurately the plant consumption. SRFs and CRFs provide a more efficient, economical, and safe way to deliver nutrients to plants as they can retain nutrients in the soil for a longer period and they are available for the plants at the desired rate or concentration level (Kontárová et al., 2022). The basic concept of these fertilizers is that they release nutrients to plants over an extended period, more efficiently than classic-release fertilizers (Khunkeaw et al., 2012), to synchronize with the nutrient demand curve of a crop during a production cycle. This can meet the crop nutrient demand for the entire season through a single application, involving savings in spreading costs (Shaviv, 2020). Thus, one-time fertilization with controlled-released fertilizer (CRF) is a promising way for reducing labor cost, increasing nitrogen use efficiency (NUE) and alleviating environmental pollution (Cui et al., 2022).

Slow-release nitrogen fertilizer (SRNF) can potentially increase crop production, improve NUE, reduce fertilizer nutrient loss, leaching and denitrification in the soil, and significantly reduce apparent nitrogen loss in the 0-90 cm soil layer (Bahar et al., 2019; Zheng et al., 2017; Feng et al., 2021), and thus reducing the environmental challenges in agro-ecosystem (Ghafoor et al., 2022). The goal in developing SRNF is having N release rated to the requirement of growing crop, thereby reducing the loss of applied fertilizer N, and increasing the NUE (Bahar et al., 2019).

Limitations to wide use in farms of SRFs and CRFs are determined by high product costs, low efficiency of early products and the use of petroleum-based synthetic polymers with uncertain environmental impact (Vejan et al., 2021). These types of fertilizers are much more expensive than classical fertilizers which makes their initial market smaller targeted to higher income crops, thus naturally the first applications have been in horticulture on small plots, and in hybrid seed maize production where income premiums allow for top tier technology usage. Unfortunately, the total use of SRFs and CRFs is much smaller than the total amount of fertilizers used globally (Bahar et al., 2019). Despite their continuous development, SRFs and CRFs still have few supporters in Europe, primarily due to their high prices (Wesołowska et al., 2021).

Maize (*Zea mays* L.) is the second most produced crop on Earth with a great flexibility of ecological growing environment. Giving its great importance, maize has been one of the main crops researched and improved genetically to unleash its production potential and to counter environmental challenges.

Hybrid maize seed since its introduction in large scale agriculture in 1920's led to 3 times

increase in yield compared to non-hybrid varieties (Duvick, 2005), due to heterozis effect, increased stress resistance, increased plant populations, and better use of fertilizers. Therefore, hybrid maize is rightly considered a big part of the green revolution as described by Norman Borlaug. The commercial production of hybrid maize seed requires technical information on agronomic management that allows obtaining the maximum yield of high-quality seed (Majeed et al., 2023). Therefore, maize seed is produced in the most technologically advanced farms requiring specific know-how and machines to obtain valuable hybrid seeds.

Fertilizers, especially nitrogen, are indispensable for achieving sufficient yields of maize, as new hybrids that can support high plant populations need high amounts of fertilizers. One of the biggest yield limiting factors after water in maize crops is fertilization, which needs to become more efficient to sustain high request of nutrients, at precise moments in time (Jeffrey and Gyles, 2003). Research has underlined that fertilization is essential for maize plant development with direct positive effect in plant height, stem thickness, foliage development, total leaf area and leaf area index, final dry matter development and yield (Law-Ogbomo and Law-Ogbomo, 2009). Maize requires high quantities of nitrogen (Minev et al., 2019), significantly increasing production costs (Zanão Júnior et al., 2019) and it must be underlined that the optimization of nitrogen fertilization in maize cannot be done on a global scale but must be done for specific soil and climatic conditions (Matev and Minev, 2020). Therefore, nitrogen use efficiency has been a subject of attention for a long time in agronomic science, due to its foremost influence on maize yield (Barşon et al., 2021).

The aim of the paper is to present the results of the performed research focused on analyzing and comparing the new types of fertilizers (slow-release and controlled-release fertilizers) to classical ones, in terms of yield (i), and phenological (ii) and phenotypical development of plants (iii) in hybrid seed maize production under irrigated conditions, as well as in researching the optimum time of application (iv) for each kind of the studied fertilizer.

MATERIALS AND METHODS

Research has been conducted in field experiments under irrigation conditions over three years (2020, 2021, and 2022) in the pedo-climatic conditions of the Moldavian plain, near Prut River in NE Romania (47.5188° N, 27.4490° E), in Bivolari commune, Iasi County. Research was performed in a farm specialized in hybrid seed production at maize and other field crops, respectively SC Integrasem S.R.L. which is producing hybrid seed of maize on a surface of 2,500 ha.

Field research was organized as subdivided experimentation plots with 4 replications and the following experimental factors:

- Factor A – nitrogen fertilizer product, with 5 treatments:
  - a1 = Control 1 – no added nitrogen fertilizer in spring;
  - a2 = Control 2 – no added nitrogen fertilizer in spring, but with complex fertilizers added at seedbed preparation;
  - a3 = ammonium nitrate added to Control 2, as classical nitrogen fertilizer (CNF);
  - a4 = Urea NG added to Control 2, as slow-release nitrogen fertilizer (SRNF);
  - a5 = Multicote (4) 34-0-7 added to Control 2, as control-release nitrogen fertilizer (CRNF).
- Factor B – fertilizer rate split between seedbed preparation and BBCH 16, respectively at growth stage of maize plants of 6 leaves unfolded, codified by BBCH (Meier, 2001), with 4 treatments:
  - b1 = 100% at seedbed preparation (SbP);
  - b2 = 50% at SbP + 50% at BBCH 16 side-dressing;
  - b3 = 25% at SbP + 75% at BBCH 16 side-dressing;
  - b4 = 100% at BBCH 16 side-dressing.

Control 1 variant received only 100 kg/ha of ammonium nitrate applied pre-ploughing in the autumn of the preceding year with the aim of sustaining the preceding crop residue decomposition. For this experimental variant, there was no fertilizer added in the spring.

Control 2 variant is the basic fertilization scheme used by the seed multiplier SC Integrasem S.R.L. and approved by the parent company of the inbred lines. Control 2 variant consisted of fertilization with 100 kg/ha of

ammonium nitrate applied pre-ploughing in the autumn of the preceding year (as in the case of Control 1), plus 300 kg/ha of complex fertilizer 14:14:14+7SO_3+4MgO applied at seedbed preparation and 150 kg/ha of complex fertilizer 20:20:0+0.5Zn applied also at seedbed preparation. These fertilizers assured the following rates of nutrients: 105.5 kg/ha N; 72 kg/ha P_2O_5; 42 kg/ha K_2O; 21 kg/ha SO_3; 12 kg/ha MgO; 0.75 kg/ha Zn.

The nitrogen fertilizer products used in the performed research (ammonium nitrate, urea NG, and Multicote (4) 34-0-7) assured each a nitrogen rate of 67 kg/ha, this providing for all the experimental variants except Control 1 and Control 2 the same total nitrogen rate of 172.5 kg/ha (including in this amount the nitrogen applied at seedbed preparation through complex fertilizers).

Ammonium nitrate (33.5% N) is a classical fertilizer with rapid release of nitrogen. Urea NG (46% N) has its grains coated with a Neem extract (N-Guard Oil) film which assures a slow-release of nitrogen and consequently allows plants to absorb the nitrogen gradually over a longer period. Multicote (4) 34-0-7 (34% N and 7% K_2O) is a control-release fertilizer which is releasing nutrients over 4 months at soil temperature of 21°C.

The nitrogen application at BBCH 16 growth stage and side-dressing was chosen taking into account the maize has a sigmoidal nitrogen uptake pattern with a turning point at BBCH 16 from when nitrogen consumption increases exponentially. It is considered that, no matter of application method, the mineral nutrient content stored in maize leaves at BBCH 17 stage deeply impacts maize yields. The side-dressing application with cultivator was chosen having into account to cover fertilizer granules with soil for diminishing nitrogen losses through volatilization process (Gaj et al., 2012).

The size of the experimental variant was 192 m^2, which resulted from a width of 4.8 m, meaning 8 rows with 0.6 m spacing between rows, and a length of 40 m. Among the 8 rows, 6 were female rows and 2 were male rows.

The biological material used in the 3 consecutive years of research was the male and female parental lines of commercial hybrid P9889, a FAO 350 maize hybrid with medium precocity.

The seeding parity of the male and female parental lines was 6:2, with 6 female rows and 2 male rows with the row spacing of 60 cm and 18.3 cm between plants on row. The planting ratio of 6:2 is a newer ratio introduced in Romania in 2010's. The ratio of female to male rows of 6:2 is less complicated and less costly to handle with conventional planters and harvesters. It assures the increase of the surface per hectare of female harvestable plants (75%) compared to more classical ratios like 4:2 where only 67% of the surface is covered with female plants (Wright, 1980).

The planting schedule must consider the difference in maturity between the two parental lines together with the objective of having pollen in the field for a longer period. Thus, the first planting pass requires the planting of the female and first male (one male row), and the second male (the second male row) is to be planted after 33 GDD (Growing Degree Days with the biological threshold of 10°C). It is recommended to sow male rows on at least two consecutive dates, so that the pollination period of the plants on male rows completely encompasses the receptivity period of the plants on female rows (MacRobert et al., 2014).

In terms of tillage, it was applied conventional tillage with plowing at 27 cm in autumn, followed by a disc ripper to level the soil. In spring, the seedbed preparation was done at a depth of 6 cm in the day before sowing.

Sowing time was established by determination the temperature of 10°C at seeding depth for the first stage of planting Female + Male 1, while Male 2 was planted as stated after reaching 33 GDD. Practically, sowing was performed each year in the middle of April.

Weed control is essential in hybrid seed maize crops and thus a two-step herbicide program was implemented with Adengo 465 SC (225 g/l Isoxaflutole + 90 g/l Thiencarbazone-methyl + 150 g/l Cyprosulfamide - safener) 0.35 l/ha applied at BBCH 12 to control dicotyledonous and annual monocot weeds. Late post-emergence herbicide application of Laudis OD 66 (44 g/l Tembotrione + 22 g/l Isoxadifen-ethyl - safener) 2.25 l/ha at BBCH 18 has led to a field cleaning of weeds.

Irrigation consisted of 5 sprinkler irrigation applications of 30 mm: first irrigation was performed at BBCH 14 after herbicide

application in order to activate it and support plant development; second irrigation was performed at BBCH 18 after second herbicide application and after fertilizer application in order to make the nutrients available as soon as possible; third irrigation was performed at BBCH 63, at tassel appearance to ensure water for the high consumption period at flowering; fourth irrigation was performed during flowering in order to sustain pollination and help plants surpass the high heat during that time; fifth irrigation was performed at grain filling time, which is critical to have good thousand grain weights results helping to obtain higher yields. Irrigation was applied with automated equipment with central pivot.

The crop technology was designed to assure optimum growing conditions for maize plants concerning nutrition, water supply and plant protection.

The soil on which the experiments were conducted is a very fertile one from the class of Chernozems. Ph is 7.52, total nitrogen content is 15.09 mg/kg, phosphorus content is 21.00 mg/kg, and potassium content is 228.54 kg/kg, according to soil analyses performed in 2022.

Under the Köppen climate classification the location of the study is within a warm-summer humid continental climate (Dfb). The multi-annual mean temperature is 11.16ºC and multi-annual precipitations amount to 523.2 mm, from data collected for the past 61 years by the local meteorological station. Location altitude is 160 m above sea level.

Comparing the historical averages and the averages for 3 years of research, one can observe a consistent pattern of warming especially in the cold months between October and March with an average increase of 3ºC for this period (Table 1). In terms of annual precipitation regime, the impact is more important with a decrease of 41.8 mm which shows deteriorating conditions for crop growing especially in the case of maize where the average decrease of 36 mm in July is occurring during the critical phase before flowering where maximum water availability is required (Table 2).

During the 3 years of research, the variability of conditions ensures that the field experiments were conducted under different meteorological conditions which increase the general character of the obtained results (Table 1 and Table 2).

Table 1. Monthly temperature and historical comparison

Year/Months	Temperature - Monthly Average (°C)												Annual
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Mean
2020	1.1	4.1	7.2	11.2	14.6	21.9	22.3	23.5	19.6	14.4	5.3	2.4	12.3
2021	0.2	-1.6	3.7	8.6	15.4	20.9	24.1	21.3	15.7	9.9	6.6	0.0	10.4
2022	0.5	3.8	3.7	10.4	16.8	22.1	23.6	23.5	16.1	12.3	5.7	1.0	11.6
Average (A)	0.6	2.1	4.9	10.1	15.6	21.6	23.3	22.8	17.1	12.2	5.9	1.1	11.4
Historical Average (HA)	-3.8	-2.0	2.9	10.8	18.9	23.3	24.4	23.5	16.8	9.5	2.8	-1.1	10.5
Difference (A – HA)	4.4	4.1	2.0	-0.7	-3.3	-1.7	-1.1	-0.7	0.3	2.7	3.1	2.2	0.9

Table 2. Precipitation regime and historical comparison

Year/Months	Rainfall - Monthly Average (mm)												Annual
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Sum
2020	3.4	32.0	15.7	1.8	132.0	98.4	30.4	5.8	26.3	80.1	13.0	41.0	479.9
2021	19.1	28.2	45.4	40.2	67.4	109.4	40.5	132.8	6.6	3.5	10.3	61.2	564.6
2022	9.1	10.8	8.4	52.7	21.5	35.1	28.4	61.3	85.6	13.5	54.4	18.9	399.7
Average (A)	10.5	23.7	23.2	31.6	73.6	81.0	33.1	66.6	39.5	32.4	25.9	40.4	481.4
Historical Average (HA)	27.9	25.8	28.4	43.4	55.1	80.7	69.1	55.4	52.3	23.5	30.7	30.9	523.2
Difference (A – HA)	-17.4	-2.1	-5.2	-11.8	18.5	0.3	-36.0	11.2	-12.8	8.9	-4.8	9.5	-41.8

The year 2020 registered the highest recorded annual average of 12.3ºC and high temperatures in July and August, to which one adds the effect of pedological draught starting in the second decade of July and the whole month of August, when total precipitations amounted 5.8 mm. This climate context impacted heavily on yield establishment as there were improper conditions at maize flowering time and on pollen viability.

The year 2021 was a good year to grow maize in NE Romania as temperatures were cooler

than historical averages across the growing period which, together with 41.4 mm of extra yearly precipitation mostly in June and August, facilitated exceptional conditions at pollination time especially.

The year 2022 was the worst climatic year for maize growing in the past 60 years. Rainfall had a gross deficit of 123.5 mm with monthly averages less than 50% of the historical averages in May, June, and July which gravely impacted crop establishment and yield formation.

Phenological determinations aimed at the important dates for crop development in the case of maize for producing hybrid seeds. For crop establishment there were noted the planting date of plants on female rows and male 1 and male 2 rows and the emergence date of each inbred line. For flowering time there were noted the date when 10% of female plants were at silking stage (with stigmata visible), as well as the date when 10% of male plants on the two rows (male 1 and male 2) had tassel emerged. Also, it was noted the detasseling date and end of flowering time for each inbred line, as well as the date of harvest.

Phenotypical determinations aimed the plant development in terms of plant height and number of leaves before detaselling.

Cob harvest was done manually at grain humidity of 34%, which is ideal for cob harvesting. A number of 400 cobs were harvested per variant, i.e. 4 repetitions x 100 cobs. The grain yield was determined by shelling the grains from the cobs. Also, the yield components of the cob were determined as well as the thousand grain weight.

To determine the statistical significance of the set of primary data, obtained in the field experiments, it was used the test package from the Microsoft Excel statistical analysis extension XLSTAT. Further analysis was conducted using Fisher test to strengthen the analysis of differences between variants and control (P-value < 0.05). Dunnett test was used to compare variant by variant with the control. Tukey test was used to test for significance of all pairwise comparisons, which compares each variant against each other and against control variants to find the most statistically significant variants which are the best options to propose as recommendations for use in practice.

RESULTS AND DISCUSSIONS

Analysis of the average yield results of the 3 years of the performed research, shown in Table 3, has put into evidence the variant fertilized with slow-release Urea NG with 100% rate (67 kg/ha of nitrogen) applied at seedbed preparation, in this variant being obtained the highest average yield of 10,017.5 kg/ha, which means an increase of 47.21% compared to Control 1 variant and an increase of 20.81% compared to Control 2 variant. At the same time, 3 out of the top five yielding variants have used Urea NG as fertilizer, which highlight slow-release fertilizers efficiency in providing sufficient nutrition over the growth period of the hybrid seed maize crop and the correlation between the release of nutrients and consumption curve. Close to the best variant with slow-release fertilizer Urea NG was the variant with the control-released fertilizer Multicote (4) 34-0-7 with 50% of the rate applied at seedbed preparation and 50% of the rate applied at growth stage of BBCH 16. These two best fertilization variants registered the smallest coefficient of variation in the three experimental years, respectively 0.86% and 1.00% (Table 3). This is explained by the long nitrogen release of these fertilizers which makes the nitrogen available to be assimilated by maize plants over the whole growth period, regardless of water availability.

Ammonium nitrate variants with shorter release periods of active substance are susceptible to leaching if there is too much water due to precipitation and can cover only a part in the growing period of the plants not well correlated with their need but more with water availability in the soil. So, the best ammonium nitrate variant was the one with 100% of the rate applied at growth stage of BBCH 16 (9899.2 kg/ha), but with a high coefficient of variation (8.73%), which shows the dependence on the climatic conditions of the year. The ammonium nitrate variant with 100% of the rate applied at seedbed preparation registered the smallest yield (9467.5 kg/ha) among all the experimental variants, with a small coefficient of variation (2.84%), which shows the constancy of this variant regardless the climatic conditions of the year.

Yield performance by type of product highlights a higher average yield for slow-release fertilizer Urea NG (9840 kg/ha) compared to controlled release fertilizer Multicote (4) 34-0-7 (9721 kg/ha) followed by Ammonium Nitrate (9696 kg/ha) (Figure 1). Control variants have been clearly outperformed by all the variants with nitrogen fertilizer.

Table 3. Hybrid seed yield ranking of variants

Experimental variant	Hybrid seed yield (2020-2022)			Significance	Coefficient of Variation for 2020-2022 (%)
	Average (kg/ha)	Difference (%) compared to			
		Control 1	Control 2		
Urea NG – 100% SbP	10017.5	47.21%	20.81%	A	0.86%
Multicote (4) 34-0-7 – 50% SbP + 50% BBCH 16	9961.7	46.39%	20.14%	A	1.00%
Ammonium Nitrate – 100% BBCH 16	9899.2	45.32%	19.39%	A	8.73%
Urea NG – 50% SbP + 50% BBCH 16	9886.7	45.29%	19.24%	AB	6.00%
Urea NG – 100% BBCH 16	9767.5	43.53%	17.80%	AB	3.82%
Multicote (4) 34-0-7 – 100% BBCH 16	9750.8	43.29%	17.60%	AB	6.44%
Ammonium Nitrate – 50% SbP + 50% BBCH 16	9749.2	43.27%	17.58%	AB	1.13%
Urea NG – 25% Seedbed + 75% BBCH 16	9686.7	42.35%	16.82%	AB	8.05%
Ammonium Nitrate – 25% SeP + 75% BBCH 16	9680.0	42.25%	16.74%	AB	9.29%
Multicote (4) 34-0-7 – 25% SbP + 75% BBCH 16	9680.0	42.25%	16.74%	AB	6.44%
Multicote (4) 34-0-7 – 100% SbP	9492.5	39.49%	14.48%	AB	5.35%
Ammonium Nitrate – 100% SbP	9467.5	39.13%	14.18%	AB	2.84%
Control 2 – no nitrogen fertilizer in spring, but complex fertilizers applied at seedbed	8291.7	21.85%	-	BC	2.95%
Control 1 – no nitrogen fertilizer in spring	6805.0	-	-17.93%	C	3.65%

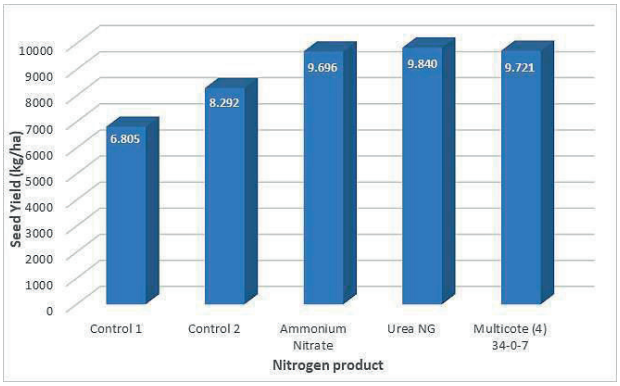


Figure 1. Average yield by specific nitrogen fertilizer type

The yield distribution of the variants shown in Figure 2 illustrates 2 clusters of yields one around the value of 9,000 kg/ha slightly above the values of the control 2 variant which varied around 8,300 kg/ha. The second main cluster around the value of 9,500 kg/ha consistent mostly of slow-release fertilizer at the top of the cluster above 10,000 kg/ha and controlled release fertilizer Multicote 34-0-7 in the upper echelon of the second cluster at around 9,600 kg/ha. It can be highlighted the fact that even though not all the variants with controlled release and slow-release fertilizers are highest performers, they are all situated in the top quartiles of yielding variants.

Statistical analysis of yield results of show that the type of fertilizer has a great importance in explaining the yields for each variant as R^2 coefficient has a high value of 0.715 (Table 4) which shows the strong relationship between fertilizer type and yield. Fisher test results $P<0.0001$ (Table 5) further strengthen the previous observation as it is a test that can show the correlation between factors and yield

even in conditions of small statistical sample sizes. The performed study is therefore statistically confirmed as being an analysis of a critical element that greatly influences final yields and the yields of slow-release and controlled-release fertilizer variants are the highest.

Using the statistical analysis tool Tuckey Test, that allows a comparison between variants and comparison to control variants, one can confidently observe that there are statistically significant differences between 4 tested variants and the rest of tested variants (Table 6).

The highest yielding variant (10,017.5 kg/ha) was Urea NG with the entire rate applied at seedbed preparation (Figure 3), which is consistent with its release pattern over 4 months which covers high N consumption stages especially between BBCH 16 and flowering. This highlights the good slow-release mechanism of this product which is correlated with maize nitrogen consumption curve. An alternative option when using Urea NG is splitting the application to 50% at seedbed preparation and 50% at BBCH 18. But, even though significant increases in yield can be observed in the case of 50/50 application of Urea NG, this option had a lower yield that classical, cheaper, fertilizer ammonium nitrate applied 100% at BBCH 16 growing stage, and the farmer will lose the economic benefit of having one less pass in the field.

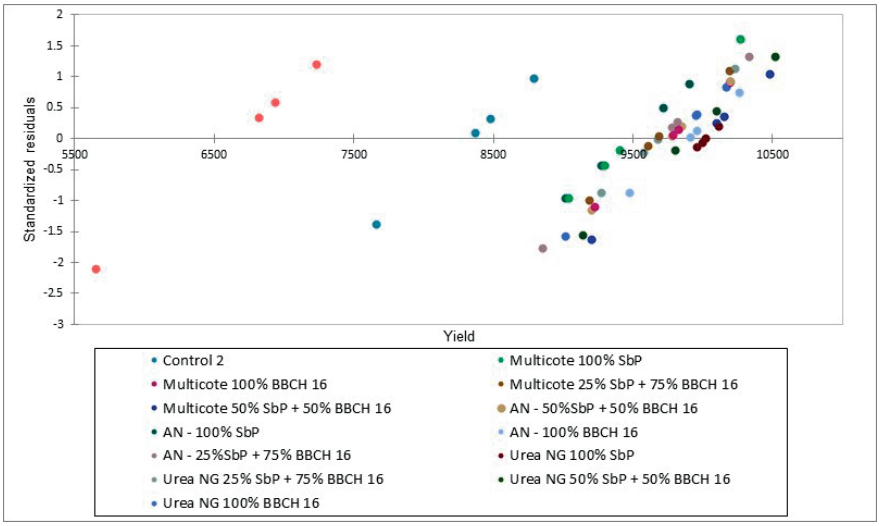


Figure 2. Yield distribution of variants

Table 4. Goodness of fit R^2

Observations	56
Sum of weights	56
DF	42
R^2	0.715
Adjusted R^2	0.627

Table 5. Fisher test values

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	13	39027419.246	3002109.173	8.103	**<0.0001**
Error	42	15561313.889	370507.474		
Corrected Total	55	54588733.135			

Table 6. Tukey test multi comparison test for hybrid seed yields according to moment of fertilizer application

Category	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups		
Urea NG – 100% SbP	10017.500	304.347	9403.304	10631.696	A		
Multicote (4) 34-0-7 – 50% SbP + 50% BBCH 16	9961.700	304.347	9347.470	10575.863	A		
Ammonium Nitrate – 100% BBCH 16	9899.200	304.347	9284.970	10513.363	A		
Urea NG – 50% SbP + 50% BBCH 16	9886.700	304.347	9272.470	10500.863	A		
Urea NG – 100% BBCH 16	9767.500	304.347	9153.304	10381.696	A	B	
Ammonium Nitrate – 50% SbP + 50% BBCH 16	9750.800	304.347	9136.637	10365.030	A	B	
Urea NG – 25% SbP + 75% BBCH 16	9749.200	304.347	9134.970	10363.363	A	B	
Multicote (4) 34-0-7 – 100% BBCH 16	9686.700	304.347	9072.480	10300.863	A	B	
Multicote (4) 34-0-7 – 25% SbP + 75% BBCH 16	9680.000	304.347	9065.804	10294.196	A	B	
Ammonium Nitrate – 25% SbP + 75% BBCH 16	9680.000	304.347	9065.804	10294.196	A	B	
Multicote (4) 34-0-7 – 100% SbP	9492.500	304.347	8878.304	10106.696	A	B	
Ammonium Nitrate – 100% SbP	9467.500	304.347	8853.304	10081.696	A	B	
Control 2	8291.700	304.347	7677.470	8905.863		B	C
Control 1	6805.000	304.347	6190.804	7419.196			C

Controlled release fertilizer Multicote (4) 34-0-7 was the second-best option with 9961 kg/ha with a split application 50% at seedbed preparation and 50% at BBCH 16 side-dressed which is optimal with the slower release of controlled release fertilizers through hydrophobic polymers compared to neem oil treated Urea NG (Figure 3).

Yield distribution for ammonium nitrate by split rate has clearly shown that the use of ammonium nitrate generates the highest yield when applied the integral rate at BBCH 16 (Figure 3). This confirms the fact that the pattern of nitrogen release for ammonium nitrate granules follows a curve with initial exponential growth which slows down after a month, thus ammonium nitrate is the optimal solution to be applied before the maximum growth intensity period from BBCH 16 to BBCH 61.

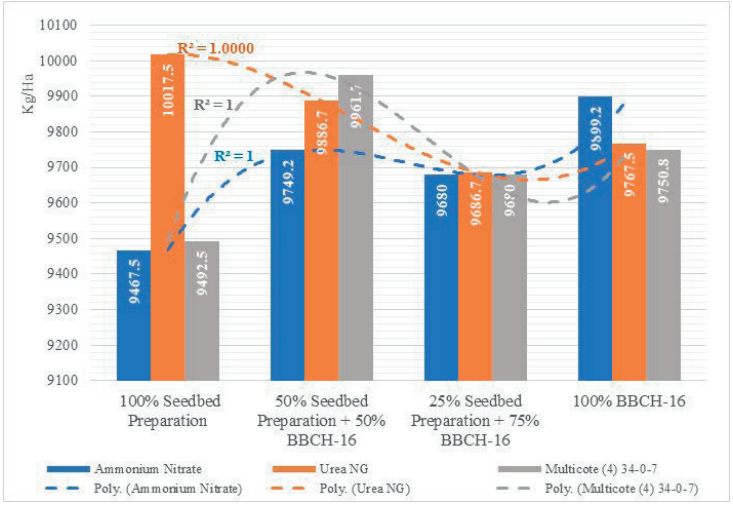


Figure 3. Average yield according to nitrogen fertilizer and moment of application

Controlled release fertilizers are best applied in 50-50 split between seedbed preparation and BBCH 16 side dressed, and slow-release fertilizers are best applied the entire rate at seedbed preparation. The use of slow release and controlled release fertilizers at the right moment of application will allow the reduction of physical quantities of fertilizer applied per hectare for the same nitrogen rate by 27% if using Urea NG and 1.5% if using Multicote 34-0-7 compared to ammonium nitrate fertilizer.

The Dunnett test (Table 7) which compares each variant with the control shows that the variant with ammonium nitrate with the entire rate applied at seedbed preparation and the one with Multicote controlled release fertilizer also applied the entire rate at seedbed preparation have not exhibited statistically significant differences compared to the control. This statistically significant discovery is consistent with the release curve of the two types of fertilizer. Thus, ammonium nitrate with nitrate and ammonium nitrogen is applied too early and is prone to leaching, the performed research being under irrigation condition, and will not be available for later stage development of maize plants. Concerning Multicote fertilizer, the hydrophobic coating will lead to a very slow release of nitrogen in initial stages of vegetation which will lead to poor crop establishment which plagues through all the future growth stages.

Phenotypical determinations conducted over the growth cycle for each of the 3 years have produced results that sustain the findings regarding yields. The variants that exhibited significant statistical differences compared to all other variants such as Urea NG with the entire rate applied at seedbed preparation, Multicote 50-50 split and Ammonium Nitrate with the entire rate applied at BBCH 16, all have resulted in exceptional phenotypical development with a plant height of over 200 cm for female inbreds before detasseling and at the same time a very good development of foliar apparatus (Table 8).

Table 7. Dunnett test control comparison test

Contrast*	Difference	Standardized difference	Critical value	Critical difference	Pr > Diff	Significant
C2 vs Urea NG – 100% SbP	-1725.833	-4.010	2.920	1256.610	0.003	Yes
C 2 vs Multicote (4) 34-0-7 – 50% SbP + 50% BBCH 16	-1670.000	-3.880	2.920	1256.610	0.004	Yes
C2 vs Ammonium Nitrate – 100% BBCH 16	-1607.500	-3.735	2.920	1256.610	0.006	Yes
C2 vs Urea NG – 50% SbP + 50% BBCH 16	-1595.000	-3.706	2.920	1256.610	0.006	Yes
C2 vs Urea NG – 100% BBCH 16	-1475.833	-3.429	2.920	1256.610	0.014	Yes
C2 vs Multicote (4) 34-0-7 – 100% BBCH 16	-1459.167	-3.390	2.920	1256.610	0.015	Yes
C2 vs Ammonium Nitrate – 50% SbP + 50% BBCH 16	-1457.500	-3.386	2.920	1256.610	0.015	Yes
C2 vs Urea NG – 25% SbP + 75% BBCH 16	-1395.000	-3.241	2.920	1256.610	0.022	Yes
C2 vs Ammonium Nitrate – 25% SbP + 75% BBCH 16	-1388.333	-3.226	2.920	1256.610	0.023	Yes
C2 vs Multicote (4) 34-0-7 – 25% SbP + 75% BBCH 16	-1388.333	-3.226	2.920	1256.610	0.023	Yes
C2 vs Multicote (4) 34-0-7 – 100% SbP	-1200.833	-2.790	2.920	1256.610	0.068	No
C2 vs Ammonium Nitrate – 100% SbP	-1175.833	-2.732	2.920	1256.610	0.078	No
C2 vs C1	1486.667	3.454	2.920	1256.610	0.013	Yes

*SbP = Seedbed preparation; C1 – Control 1; C2 – Control 2

Table 8. Maize plant height and number of leaves per plant at detasseling according to nitrogen fertilizer and moment of application

Nitrogen fertilizer		Plant height at detasseling (cm)					Number of leaves at detasseling				
Fertilizer product	Fertilizer rate split (kg/ha)	2020	2021	2022	Average	Significance	2020	2021	2022	Average	Significance
Control 1	–	179	185	173	179	C	13	15	12	13	D
Control 2	–	181	188	176	182	BC	15	16	14	15	C
Ammonium nitrate	100% SbP	184	192	177	184	B	15	16	15	15	C
	50% SbP + 50% BBCH 16	189	197	179	188	B	16	17	14	16	BC
	25% SbP + 75% BBCH 16	186	195	178	186	B	16	17	15	16	BC
	100% BBCH 16	200	207	191	200	A	17	18	15	17	AB
Urea NG	100% SbP	206	213	195	205	A	18	19	16	18	A
	50% SbP + 50% BBCH 16	198	206	190	198	A	17	18	16	17	B
	25% SbP + 75% BBCH 16	196	203	189	196	AB	16	18	15	16	BC
	100% BBCH 16	187	195	180	187	AB	16	17	15	16	BC
Multicote (4) 34-0-7	100% SbP	186	196	177	186	AB	15	18	14	16	BC
	50% SbP + 50% BBCH 16	203	210	193	203	A	17	18	15	17	AB
	25% SbP + 75% BBCH 16	194	200	186	193	AB	15	18	15	16	BC
	100% BBCH 16	195	203	188	195	AB	17	18	16	17	B

Urea NG applied pre-planting (at seedbed preparation) resulted in the highest number of leaves (18 leaves) followed by ammonium nitrate applied at BBCH 16 and Multicote 50-50 split. Thus, slow-release fertilizers with the entire rate applied at seedbed preparation, controlled release fertilizers applied 50% at seedbed preparation and 50% at BBCH 16, and classic fertilizers with the entire rate applied at BBCH 16 are the best options for optimum phenotypical development of hybrid maize seed crops.

Maize is a monoecious plant with a tendency towards protandrous development, especially under high stress conditions such as draught and dry air, thus the anthers of the male tassel open about 3 days before the emergence of female stigmata.

Field evaluations show that the average time between flowering of male 1 tassel and female stigmata emergence was 4 days for most of the variants. The 4 variants that proved to be superior in terms of yield were also superior in terms of flowering gap (Table 9). Urea NG applied at seedbed or 50/50 split between seedbed and BBCH 16, Multicote applied in a 50/50 split, together with ammonium nitrate applied at BBCH 16 had an optimum flowering gap of 3 days between male and female inbreds.

Irrigation performed in all the three experimental years during flowering sustained pollination and helped plants surpass the high heat during that time. This is important knowing that high temperatures in this phase increase the gap between the appearance of tassel and that of silks, and at a temperature of 28-30°C, the viability of the pollen decreases (Mircea et al., 2021).

The number of grains per cob depends on the type of nitrogen fertilizer (Szulc et al., 2023). The optimum flowering gap leads on average to an extra grain per cob as better pollination was realized due to the extra day of pollen availability. This extra grain as a yield formation element had an impact on final yield as exhibited by data presented.

Table 9. Flowering gap and number of grains per row according to nitrogen fertilizer type

Nitrogen fertilizer		Flowering Gap (days)					Number of grains per cob				
Fertilizer product	Fertilizer rate split	2020	2021	2022	Average	Significance	2020	2021	2022	Average	Significance
Control 1	–	5	4	6	5	C	195	223	212	210	C
Control 2	–	4	3	4	4	B	217	238	217	224	BC
Ammonium nitrate	100% SbP	4	4	5	4	B	224	231	217	224	BC
	50% SbP + 50% BBCH 16	4	4	5	4	B	224	273	217	238	AB
	25% SbP + 75% BBCH 16	4	4	5	4	B	224	273	217	238	AB
	100% BBCH 16	3	3	4	3	A	221	311	224	252	A
Urea NG	100% SbP	3	3	4	3	A	231	302	223	252	A
	50% SbP + 50% BBCH 16	3	3	4	3	A	223	310	223	252	A
	25% SbP + 75% BBCH 16	4	3	5	4	B	231	259	224	238	AB
	100% BBCH 16	4	4	5	4	B	224	231	224	238	AB
Multicote (4) 34-0-7	100% SbP	4	4	5	4	B	223	273	218	238	AB
	50% SbP + 50% BBCH 16	3	3	4	3	A	231	302	223	252	A
	25% SbP + 75% BBCH 16	4	3	4	4	B	223	274	217	238	AB
	100% BBCH 16	4	3	4	4	B	223	274	217	238	AB

The finding that the use of Urea NG used at seedbed preparation is the most productive option is in connection with a study of Zhang et al. (2019) who highlighted that the use of controlled release urea has resulted in an increase of maize yield by 5.3% as well as increased nitrogen use efficiency by 24.1%, and at the same time it meant a reduction in total nitrous oxide (N_2O) emission, N leaching as well as ammonia (NH_3) volatilization by 23.8%, 27.1% and 39.4%, respectively.

The results showing ammonium nitrate applied through side-dressing at BBCH 16 stage as a significant yield increasing variant are consistent with those obtained by Bășa et al. (2016) saying that nitrogen application in the growth stage of five leaves of the maize plants increased the values of the yield component and grain yield.

The findings regarding split application of controlled release fertilizer and pre-plant application of slow-release fertilizers are according to those of Arrobas et al. (2022) who found that for the same N rates, CRF and SF (stabilized fertilizers) gave similar DM yields, not dissimilar or even higher plant N nutritional status indices and N use efficiencies, than the conventional fertilizer split into two applications.

The finding of enhanced phenotypical development of variants fertilized with slow-release fertilizers can be corroborated with the findings of Li et al. (2020) where over ground parts dry weight has increased by 10.52-17.49%, for slow-release fertilizers, this being consistent with superior phenotypical development of variants where they have been used.

Crucial for consistent yields in hybrid seed maize production is achieving a synchronization called pollination nicking (Wright, 1980), between male anthers pollen shedding and emergence of female stigmata from husks, to have the highest rate of pollination of a kernel. The period between male and female flowering is vital because pollination, fertilization, and eventually grain filling and grain yield depend on it (Sirih et al., 2021). It is necessary to monitor the accumulated GDD to detect on time temperature changes that may modify days to flowering, the detasseling schedule, as well as the grain-filling period duration, and therefore effective seed yield improvement (Sirih et al., 2021). Therefore, an essential element to be observed when considering technological changes is their effect on nicking. For hybrid seed maize crops it is important that the carefully sought after flowering synchronization between female and male inbred is not perturbed by any of the changes made to fertilization schemes.

The length of pollen release period observed in our study is confirmed with that of Chassaigne-Ricciulli et al. (2021) who found that both the mean period of pollen release of the male inbred lines as well as the period of exposure of stigmas of the female single crosses were 4 to 5 days.

The better development of yielding elements in variants with slow release urea, applied at seedbed preparation, and controlled release fertilizer, evenly split between seedbed preparation and BBCH 16, is consistent with Li et al. (2020) which found that compared with common urea, grain number per ear, 1000-grain weight and yield of maize of the treatments with controlled-release urea were overall significantly increased, among which the yield was significantly increased by 14.81-25.84%.

It has to be considered the economic benefit of reducing one cultivation pass in the case of variants with all fertilizer applied at seedbed preparation or in vegetation period which will bring a decrease in cost per hectare (20 Euro/ha on average in the conditions of the performed research). At the same time a reduction of the number of passes on the soil will have a positive impact on soil compaction.

Logistic costs are greatly reduced as if one uses the highest yielding variant in practice, such as Urea NG all applied at seedbed preparation, which need 30% less commercial product in weight, and 1.5% less than the use of controlled release Multicote 34-0-7. As the performed study showed, the use of slow-release fertilizers can be applied as a pre-planting, production costs can be reduced, eliminating the need for multiple applications of soluble nitrogen fertilizers (Abd El-Azeiz et al., 2021).

CONCLUSIONS

The highest average hybrid grain yield at maize (10,017 kg/ha) were obtained in the case of variant fertilized with slow-release Urea NG with the whole rate (67 kg nitrogen/ha) applied at seedbed preparation and in the case of variant fertilized with control-released fertilizer Multicote (4) 34-0-7 50-50 split between seedbed preparation and BBCH 16 side dressed (9961 kg/ha).

The obtained results sustain the use of slow release and controlled release fertilizers in hybrid seed maize production, which will lead to better yields compared to classical fertilization options, no negative impact on synchronization at pollination time, lower quantities of physical fertilizer product to be used, and reduced number of passes in the field. Practically, all these make the use of this

type of fertilizers desirable in hybrid seed maize production.

The best moment of application of nitrogen fertilizers in maize seed production differs according to fertilizer type. Urea NG or other similar slow-release fertilizers which use neem extract as a nitrification inhibitor are best applied at seedbed preparation. Controlled release fertilizers such as Multicote 34-0-7 are best applied 50% at seedbed preparation and 50% at BBCH 16 with cultivation. Ammonium nitrate should be applied in the vegetation period (BBCH 16) with cultivation.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

WHEAT FLAG LEAF MORPHOANATOMICAL CHARACTERISTICS AND GRAIN YIELD COMPONENTS UNDER FIELD CONDITIONS

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Abstract

Drought induces morphological changes in plants, and among these, certain characteristics can be identified as markers of drought tolerance or resistance. The identification of drought resistance morphostructural markers can facilitate the selection of tolerant genotypes with increased productivity. Our aim was to establish a reliable and reproducible set of drought adaptation markers for Izvor and F628 wheat varieties and their DH lines, A1-3, A1-20, A1-65, A1-66, A1- 72, A2-92, A2-255 and B1-16 under field conditions. For this purpose, different leaf morphoanatomical parameters were compared with field productivity parameters. Although other studies associate these characteristics with drought tolerance, in our research most of the studied flag leaf morphological and anatomical traits were not correlated with main spike productivity or the Drought Tolerance Index (DTI). Stomatal density was the only characteristic that showed a significant correlation with the DTI, thus being considered a reliable marker for drought tolerant genotypes with increased productivity.

Key words*: wheat flag leaf, morphoanatomic characteristic, grain yield, drought resistance markers.*

INTRODUCTION

Drought is an increasingly major challenge due to continuously changing climate conditions, causing worldwide crop yield losses. Plants can undergo morphological, physiological, and biochemical adaptations to drought conditions to overcome the effects of this abiotic stress. More specifically, plants can adjust their growth, reduce resource utilization, decrease the transpiration rate, activate the antioxidant system, or decrease the chlorophyll content (Seleiman et al., 2021). Among the morphological changes in plants, some characteristics could be identified and used as markers for tolerant genotypes discrimination and for developing new varieties with increased productivity under drought stress conditions. The leaves, the main organs involved in transpiration and photosynthesis processes, are the most sensitive to hydric stress and, thus, prone to morphoanatomical changes. The analysis of their morphoanatomical characteristics can directly reflect the response to drought stress (Mehri et al., 2009; Schaller & Paschold 2009; Zhang et al., 2015).

In dicotyledons, the main anatomical features of the leaf structure associated with drought resistance potential mentioned in the literature were the thickness of the epidermal cuticles, a well-developed transport system, small size of the stomata (Ennajeh et al., 2010), leaf thickness, the thickness of the palisade and spongy tissue (Li et al., 2022), the thickness and compaction of the mesophyll tissue, the thickness of the spongy tissue, the thickness of the upper epidermis and especially the palisade tissue/spongy tissue ratio (Hu et al., 2022), the density of stomata and trichomes (Ennajeh et al., 2010). Additionally, stomatal characteristics play a crucial role in drought stress tolerance and resistance. Stomata play a fundamental role in CO_2 uptake and water use, being key factors for efficient water use. Stomata can regulate the intensity of gas exchange through the opening of the ostiole. Besides stomatal opening, size and density are important parameters for hydric deficit tolerance/resistance (Bucher et al., 2017).

Despite the abundance of studies that approaches monocots' drought tolerance, the data on morphoanatomical leaf characteristics and their influence on drought tolerance in this group of plants are scarce, the availability of these type of data being limited (El-Afry et al., 2012; Ouyang et al., 2017; Terletskaya & Kurmanbayeva, 2017; Ghafoor, 2019). This study aimed to investigate some of the flag leaf morphoanatomical parameters as drought tolerance markers for Izvor and F628 wheat varieties and their DH lines under field conditions. For this purpose, different leaf morphoanatomical parameters were compared with field productivity parameters. The correlations between these characteristics and yield components could provide a better insight into the direct effects of morphoanatomical characteristics on crop productivity in variable field drought conditions.

MATERIALS AND METHODS

Plant material

Plant material was represented by wheat plants grown in field conditions. The two wheat genotypes, Izvor and F628 and eight doubled haploid (DH) lines (A1-3, A1-20, A1-65, A1-66, A1- 72, A2-92, A2-255 and B1-16). DH lines were obtained from the cross F628/Izvor, using the protocol described by Giura (2011).

The cultivar Izvor was noticed for its performance in several dry years (Mustăţea et al., 2009; Marinciu et al., 2021), which was attributed mainly to superior osmotic adjustment (Bănică et al., 2008; Ciucă et al., 2009).

All genotypes were planted in the field at the National Research & Development Institute – Fundulea, Romania (44°30'N, 24°10'E) in 2019-2020 and 2020-2021, on chernozem soil, three replicates, in rows spaced at 30 cm.

Morphoanatomical analyses

The plant material used for morphoanatomical analyses was represented by completely developed flag leaves from the 2019-2020 season. The stomata characteristics were determined using the imprinting method. Completely developed flag leaves were detached from three different plants and were used to obtain abaxial leaf imprints. Nail polish was applied to the mid-point between the leaf extremity and the central vein at the middle of longitudinal leaf axis. The imprints were then transferred on glass slides using adhesive tape.

The leaf thickness was measured on five completely developed flag leaf sections made on ethanol-fixed material using the MT.5503 manual microtome. The sections were used as fresh preparations or stained with 0.2% Nil Blue A. The imprints and the sections were observed and imaged under an optical microscope (Imager M2 Axio Zeiss, Germany).

Morphoanatomical parameters

The analysed morphological parameters were stomata arrangement pattern, stomatal density, stomatal size, flag leaf thickness, bulliform cell height (BCH), abaxial (ABE) and adaxial epidermal thickness (ADE).

Stomata density was determined by counting the stomata of eight 1 mm^2 areas from each leaf. Stomata size was determined by measuring the length and the width of five guard cells from each leaf.

The leaf thickness was measured in five leaf areas: at the central vein point (midvein – MvT), on both sides of midvein vicinity areas (MvVT), in the middle of first bulliform cells from the midvein (FBT), at the second stomata row from the midvein (SST), and at the first secondary vein (FSV). All measured data were collected using AxioVision Rel. 4.8 Software.

Grain yield parameters

The registered grain yield parameters were the number of grains/spike (GNS) and the grains weight/spike (GWS) in the dry 2019-2020 season and the favourable 2020-2021 season. For GNS and GWS determination, 30 spikes of each genotype were used.

For the estimation of the abiotic stress response, the Drought Tolerance Index (DTI) was calculated as the ratio between the grain weight per spike measured in 2021 (the season with more favourable conditions for plant development) and in 2020 (the season with lowest average values):

$$DTI = GWS\ 2021/GWS\ 2020$$

The weather data were registered by Fundulea National Agricultural Research and Development Institute's meteorologic station.

The 2019-2020 season was drier and generally warmer than long-term average for each month. The 42 mm deficit from September, already put soil moisture content on an unfavuorable trend for crops. The rainfall deficit for December 2019

to April 2020 exceeded 114 mm, and this had a negative influence on both winter and spring crops. Even if the reduced rains from May-June (summing up 112 mm together) alleviated temporary the water stress, it increased again in July 2020 when precipitation was 34 mm (37 mm less than long term average) The months November 2019 -March 2020 were (each) with more than +3°C warmer than long-term average. Only May 2020 was as warm as the long-term average (LTA); all other months exceeded their long-term monthly averages. The warmer weather, combined with dry winds (especially in April 2020), significantly increased potential evapotranspiration and created several periods of heat stress conditions, in addition to water stress. During September 2020 - August 2021 period, the weather was rather unstable, the periods with heavy rains and strong winds alternating with prolonged drouthy periods.

During that vegetation season, there were six months (October and November 2020, February, April, May, and July 2021) with precipitation below the long-term average. Rains from June (21 rainy days and high nebulosity) contributed to a lesser extent to the grain filling of winter cereals. April 2021 was the only month cooler than usual (with -1.6°C) during that vegetation season. The period September 2020 - February 2021, was warmer than usual in average +3°C. Even if the cold hardening conditions were unsatisfactory, the winter crops managed to avoid winter kill situations because the minimal temperature didn't drop below 11.9°C (19th of January 2021), and the snow cover provided good thermal insulation.

Statistical analysis

The data were statistically processed, the correlations were calculated, and the significance test available in Excel 2019 was applied.

RESULTS AND DISCUSSIONS

Flag leaf anatomical characteristics

The flag leaf transverse sections exhibit the monocot cell organization pattern. The adaxial and abaxial epidermis of the leaf enclose the mesophyll, which is crossed by vascular tissue, resulting in areas of mesophyll alternating with areas of vascular tissue, parallel to each other.

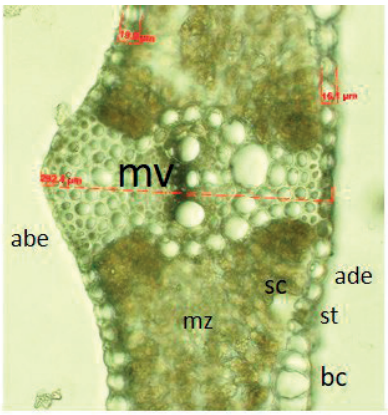


Figure 1. Anatomical structure of F628 genotype flag leaf: **ade** - adaxial epidermis, **abe** - abaxial epidermis, **mv** - midvein, **mz** - mesophyll tissue, **bc** - bulliform cells, **st** - stomata, **sc** - substomatal chamber

At the adaxial epidermis (Figure 2A) level, different cell types can be observed. The largest adaxial epidermis cells are the bulliforms, with globular-oval shape, generally being observed in groups of 3-4 cells (Figures 1 and 2 A). These are positioned in the areas between the vascular tissues. The adaxial epidermis (Figure 2 A) is composed mainly by small, flat cylindrical cells, regularly alternating with stomata. Stomata usually appear flanking the group of bulliform cells. Above the veins are cylindrical cells with thickened walls.

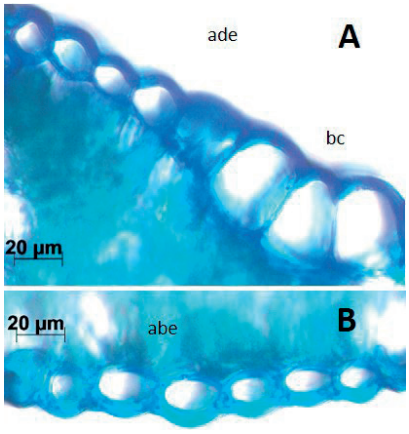


Figure 2. Details of the adaxial and abaxial epidermis of A2-255 genotype flag leaves (A, B): **ade** - adaxial epidermis, **abe** - abxial epidermis, **bc** - bulliform cells

In the less complex abaxial epidermis (Figures 2 B and 3 A) predominate flat cells with thickened walls, smaller than the epidermal cells observed

on the adaxial side. Between the vascular tissue areas, stomata were observed. As in case of adaxial epidermis, the abaxial epidermis presents trichomes.

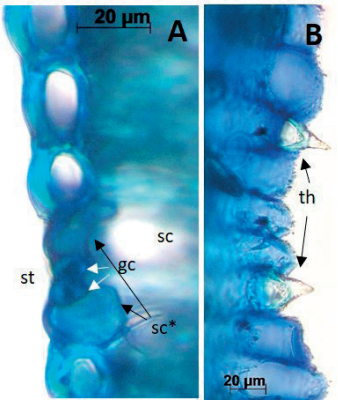


Figure 3. Details of the adaxial and abaxial epidermis of A2-255 (A) and B1-16 (B) genotype flag leaves: **ade** - adaxial epidermis, **abe** - abaxial epidermis, **bc** - bulliform cells, **th** - trichome, **st** - stomata, **gc** - guard cells, **sc*** – subsidiary cells, **sc** - substomatal chamber

Stomatal arrangement pattern

In grasses stomata consist of two dumbbell shaped guard cells positioned in front of each other, which are flanked by two subsidiary cells, together forming the stomatal complex (Figure 3 A).

Our observations show that stomata are usually arranged in parallel simple rows (Figure 4 A) and sometimes in double, intercalary rows (Figure 4 B) along the veins. Stomata alternate in the same row, usually with one interstomatal cell of different sizes (Figure 4 A). All the variants observed in this study presented spaced stomata, being separated by at least one epidermal cell of different sizes. The stomatal arrangement is essential for its proper functioning, being considered that a stomata should be separated by at least one epidermal cell from all neighbouring stomata (Dow et al., 2014). Rapid ionic exchange is needed for guard cells' proper function, and this condition is provided by the epidermal cells surrounding stomata (Dow et al., 2014).

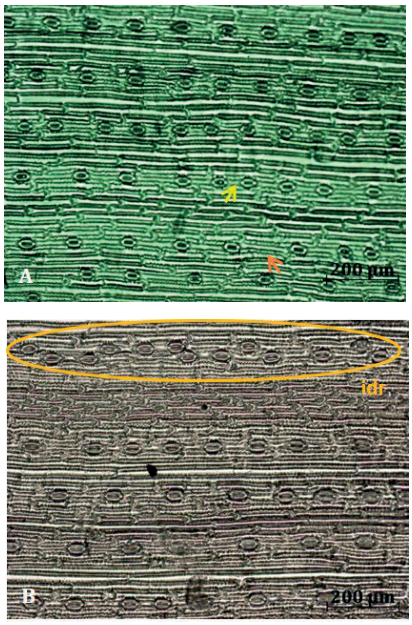


Figure 4. Stomatal arrangement patterns in Izvor (A) and F628 (B) wheat genotypes: **idr** - intercalary double rows; **yellow arrow** - short interstomatal cell; **brown arrow** - long interstomatal cell

Stomatal density and size

The stomatal density was relatively uniform among the studied genotypes and varies between 41.68 and 57.43/mm^2 (Table 1). However, the genotype with the highest stomatal density, B1-16, had significantly higher stomatal density comparing with its genitor F628 and A1-3 ($p< 0.05$). The A1-3 variant had the lowest value, being significantly different comparing to A1-66, A1-72, A2-92, A2-255 and B1-16 ($p<0.05$).

Table 1. Stomatal density and stomatal area in F628 and Izvor parental lines and their DH descendants

Genotype	Stomatal density/ mm^2	Stomatal area µm^2
F628	47.26±3.60	346.08±2.01
Izvor	50.50±4.60	251.06±1.31
A1-20	48.76±4.73	276.91±1.22
A1-3	41.68±5.04	340.43±2.40
A1-65	50.37±3.8	408.61±0.97
A1-66	56.71±5.45	182.87±0.77
A1-72	55.28±4.78	199.32±2.18
A2-255	54.14±4.12	212.93±1.19
A2-92	55.65±4.09	266.41±2.01
B1-16	57.43±4.50	187.71±1.02

Data from scientific literature showed that generally lower stomatal density was associated with drought tolerance/resistance (Hepworth et al., 2015, Hughes et al., 2017, Cain et al., 2019, Dun et al., 2019), due to total pore surface decrease.

Our results showed that the area of stomata varies between 182.87 and 408.61 μm^2. The highest area of stomata was registered in A1-65, F628 and A1-3 genotypes, while A1-66, B1-16, A1-72 had the lowest stomata area (Table 1). Also, the resistant genotype Izvor had smaller stomata than the F628 genotype. It was advanced the idea that having smaller stomata is a desirable characteristic for hydric stress tolerant/resistant lines. Smaller stomata leave more space for other epidermal structures like trichomes, subsidiary cells (Franks & Beerling, 2009), and bulliform cells, which also have important roles in water stress resistance. Additionally, in genotypes with smaller stomata, a larger area of epidermal cells is available for the secretion of waxes and cutin, which together form the outer protective layer, the plant cuticle. Higher total cell surface area of smaller stomata compared with its volume allows faster ion fluxes across the plasma membrane and tonoplast, which results in more rapid guard cells movement and, implicitly, a faster ostiole closure (Lawson & Vialet-Chabrand, 2019).

In our study, a strong negative correlation was found between stomata density and stomata area (Figure 5). Studies on different plant species also evidenced that often there is a negative correlation between stomatal density and stomatal size, in case of genotypes with a high number of stomata their size being smaller (Hetherington & Woodward, 2003, Camargo & Marenco, 2011, Faralli et al., 2019) and vice versa. This negative correlation represents an adjustment mechanism of total stomata opening. The variation of these characteristics is not always correlated, the modifications occurring sometimes independently. In the study of Doheny-Adams et al. (2012), Arabidopsis plants grown in hydric deficiency conditions did not change their stomatal density but were observed modifications like stomatal size, guard cells area, ostiole area, and implicit gas exchange rate.

Although the genotypes with low values of stomatal conductance determined by the low stomata density and/or small stomata opening would be appropriate candidates for drought tolerance/resistance, it should be considered that the photosynthesis rate decreases with stomatal conductance increase. A low photosynthesis rate can negatively influence plant development and implicitly crop productivity.

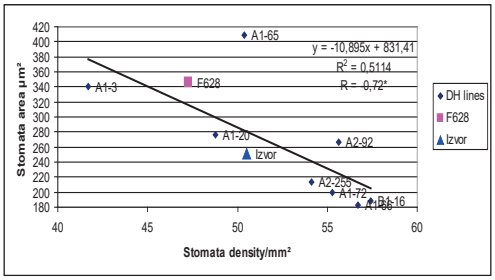


Figure 5. Correlation between stomata density/mm^2 and area (μm^2)

Bulliform cells size

Considering the longitudinal size of the bulliform cells in the transverse section, the genotypes B1-16, A1-66, A1-65, and A2-255 overcame the parental lines. Additionally, in the resistant genotype Izvor, higher values of bulliform cell size were observed compared to the F628 genotype (Table 2).

Table 2. Bulliform cell height (BCH), adaxial epidermal thickness (ADE), abaxial epidermal thickness (ABE), ratio between ADE and ABE (ADE/ABE) and difference between ADE and ABE (ADE-ABE) in F628 and Izvor parental lines and their DH descendants

Genotype	BCH (μm)	ADE (μm)	ABE (μm)	ADE/ ABE	ADE-ABE
F628	37.26 ±1.48	20.75 ±1.94	17.65 ±1.46	1,18	3,10
Izvor	41.38 ±2.42	20.13 ±1.85	18.85 ±2.57	1,07	1,28
A1-20	59.46 ±2.24	18.47 ±2.37	16.80 ±1.79	1,10	1,67
A1-3	37.47 ±2.36	22.07 ±0.88	18.5 ±0.93	0,99	-0,16
A1-65	47.47 ±2.47	20.46 ±1.64	21.88 ±2.59	1,11	1,96
A1-66	46.33 ±6.58	21.40 ±2.07	17.57 ±1.71	0,98	-0,48
A1-72	35.59 ±2.53	19.74 ±2.45	17.64 ±2.32	1,12	2,16
A2-255	48.73 ±4.44	23.43 ±1.58	22.23 ±1.34	1,33	5,79
A2-92	35.76 ±2.93	17.69 ±1.70	17.05 ±1.43	1,04	0,64
B1-16	42.56 ±4.02	19.40 ±1.36	21.20 ±2.12	0,92	-1,80

The bulliform cells, also called “motor cells” are involved in leaf movements, and play an important role in leaf curling, which is related to

resistance of plants in adverse conditions (Zheng et al. 2002, Kirkham, 2014). In drought conditions, the bulliform cells dehydrate, causing the leaf to adaxially fold and thus reducing the water loss (Kirkham, 2014; Roodt, 2021).
The size of bulliform cells can influence the speed of leaf rolling/folding, the reduction of exposed foliar area and implicitly the water loss limitation (Willick et al., 2018).

Epidermal thickness

The thickness of the epidermal layer varied between 17.62-23.34 µm for the adaxial epidermis and 16.8-22.23 µm for the abaxial epidermis (Table 2). In general, the thickness of the adaxial epidermal layer was higher than the abaxial one, with the exception of the B1-16 line. The two parental lines had very close values of adaxial epidermis thickness, and the descendants A1-66, A1-3 and A2-255 presented an increase of this parameter comparing with Izvor and F628. The variants B1-16, A1-65, and A2-255 had thicker abaxial epidermis compared with the two parental lines. Previous studies showed that anatomical characteristics like adaxial and abaxial epidermis thickness represent features that can indicate the potential resistance of different varieties to stress conditions like drought, salinity and high temperature (Terletskaya and Kurmanbayeva, 2017, Erezhetova et al., 2021, Hu et al., 2022).

Flag leaf thickness

The flag leaf thickness, measured in five different leaf areas, varied among the samples and according to the measured area (Table 3). Our results did not show significant differences in flag leaf or epidermal thickness between the Izvor drought tolerant genotype and the F628 genotype, the measured values being very close. However, Al-Maskri et al. (2013) demonstrated that modifications in leaf structural features related to drought tolerance included a thick epidermis and highly developed bulliform cells, which prevent water loss in semi-arid wheat varieties. Also, highly tolerant ecotypes of *Cenchrus ciliaris* L. showed increased epidermal thickness in leaves with well developed bulliform and decreased stomatal density on both leaf surfaces (Mansoor et al. 2019).

Table 3. Flag leaf thickness in different leaf areas: midvein - MvT, midvein vicinity areas - MvVT, first bulliform cells from the midvein - FBT, second stomata row from the midvein - SST, and first secondary vein - FSV

Genotype	MvT (µm)	MvVT (µm)	FBT (µm)	SST (µm)	FSV (µm)
F628	299.10 ±6.81	236.92 ±12.52	215.53 ±8.00	196.00 ±8.60	191.45 ±4.53
Izvor	301.57 ±38.11	255.98 ±27.62	237.21 ±21.30	201.66 ±13.70	191.26 ±10.54
A1-20	276.66 ±4.07	250.53 ±6.07	255.33 ±5.70	241.72 ±11.20	216.47 ±11.43
A1-3	314.06 ±5.07	249.23 ±8.10	249.57 ±4.00	230.73 ±8.60	223.93 ±11.16
A1-65	342.94 ±3.15	283.93 ±7.02	274.86 ±10.10	261.37 ±4.50	251.23 ±3.47
A1-66	375.30 ±5.62	287.48 ±6.26	273.73 ±9.80	243.82 ±15.00	224.50 ±3.12
A1-72	307.88 ±2.76	244.65 ±17.85	242.57 ±22.10	235.49 ±16.10	202.84 ±16.75
A2-255	392.02 ±2.13	287.84 ±9.38	269.81 ±9.60	257.86 ±12.00	218.59 ±9.27
A2-92	292.52 ±4.99	252.68 ±6.20	242.87 ±8.80	228.88 ±12.40	208.88 ±17.56
B1-16	397.00 ±8.83	322.91 ±17.39	321.74 ±10.00	294.37 ±19.50	239.54 ±10.80

In case of DH lines we observed some differences comparing with the parental lines. Regarding the MvT parameter, only two of the descendants registered lower values than the parental lines. The second measured foliar parameter MvVT showed a difference between the two parental lines F628 being outclassed by all the analysed descendants while the Izvor line only by four of the descendants. For all the other foliar parameters FBT, SST, and FSV, the parental lines registered the lowest values. The observation that for most traits, the values recorded for the DH lines were different (higher in majority) than the values of both parents, suggests the possibility of obtaining transgressions.

Correlation between morphoanatomical traits and grain yield parameters

Correlation coefficients between the studied leaf traits showed a very strong association between the thicknesses measured at different leaf parts (Table 4). This indicates that an isometric growth occurred, the proportions of the analysed leaf anatomical characteristics being maintained among the genotypes studied.
However, lower correlations were observed with epidermis thickness, suggesting a relatively independent genetic determination.

Table 4. Correlations between leaf characteristics of F628 and Izvor parental lines and their DH descendants

Analyse of traits	MvT	MvVT	FBT	SST	FSV	BCH	ADE	ABE
MvT	1							
MvVT	**0.90**	1						
FBT	**0.79**	**0.95**	1					
SST	**0.73**	**0.86**	**0.95**	1				
FSV	0.58	**0.72**	**0.80**	**0.84**	1			
BCH	**0.82**	**0.72**	0.53	0.46	0.53	1		
ADE	0.53	0.19	0.05	0.03	0.11	0.62	1	
ABE	0.49	0.45	0.47	0.29	0.41	0.31	0.38	1

Least significant correlation coefficient (P< 5%) = 0.63
Least significant correlation coefficient (5%<P< 1%) = 0.79

Table 5. Correlations between leaf anatomical traits, main spike yield components and DTI

Analyze of traits	GNS	GWS	TGW	DTI
MvT	0.31	0.27	0.15	0.29
MvVT	0.27	0.26	0.15	0.28
FBT	0.25	0.32	0.27	0.2
SST	0.32	0.46	0.47	0.27
FSV	0.27	0.33	0.32	0.15
BCH	0.02	-0.08	-0.17	0.33
ADE	0.03	-0.07	-0.14	-0.18
ABE	0.04	-0.07	-0.17	-0.26
ADE/ABE	0.02	0.05	0.07	0.11
ADE-ABE	-0.01	0.01	0.05	0.11
SD	0.05	0.15	0.19	**0.77**
SA	0.15	0.01	-0.1	-0.26
GNS	1	0.87	0.53	-0.08
GWS	0.87	1	0.88	-0.05

None of the studied leaf anatomical traits was correlated with the spike yield components, and only one correlation with DTI was significant (Table 5). These results suggest that although some anatomical characteristics related to drought stress tolerance had higher values in DH lines than in the parental genotypes, this is not necessarily transcribed in the genotype's productivity.

The only significant correlation with DTI was the one with stomata density, the genotypes with higher density showing better drought response (Figure 6). Other studies showed that genotypes with lower stomatal density have better drought tolerance capacity (Hepworth et al., 2015; Hughes et al., 2017; Cain et al., 2019; Dun et al., 2019). However, our results are in agreement with Shahinnia et al. (2016), who showed that a drought-tolerant wheat line had significantly more and smaller stomata compared with a genotype that had a significantly reduced yield under the same conditions.

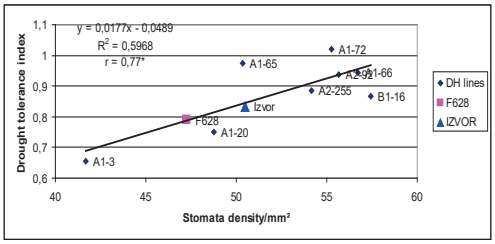


Figure 6. The strong correlation between high stomata density/mm^2 and the DTI (r> 0.5)

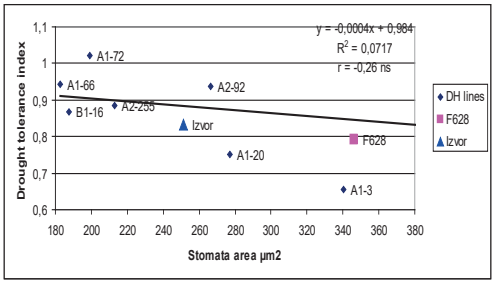


Figure 7. Relationship between stomata area (µm^2) and the DTI

As seen in Figures 6 and 7, our data showed a better association of the DTI with stomata density than with stomata area. This suggests that, in the germplasm represented by the studied genotypes, stomatal density may be a better selection criterion than stomatal area.

Overall, the grain yield performances of the studied genotypes could not be significantly sustained by the analyzed morphoanatomical traits, except for stomatal density. This observation suggests the potential involvement of additional mechanisms for drought tolerance and grain yield productivity in these genotypes.

CONCLUSIONS

The related genotypes included in this study exhibited variability in the morphological and anatomical traits of the flag leaf. For most traits, the DH lines obtained from the cross F628/Izvor had values that were, in the majority, higher than those of the parents, suggesting a high frequency of transgressions.

Thickness measurements at different parts of the leaf were strongly correlated, while the correlations of these characteristics with epidermis thickness were lower and mostly non-significant.

Most of the studied morphological and anatomical traits of the flag leaf were not correlated with main spike productivity or the DTI, suggesting that these foliar parameters play a relatively small role in determining the difference in yield or drought response of the analyzed genotypes.

Stomata density was negatively and significantly correlated with stomata area, but only stomata density showed a significant correlation with the DTI. Thus, stomatal density can be considered a reliable marker for drought tolerance in productive genotypes.

Although these results were obtained with only a small number of genotypes, they may help in the search for efficient selection criteria in breeding for drought tolerant genotypes.

ACKNOWLEDGEMENTS

This research was funded by ADER Proiect No. 321/2019, Ministry of Agriculture and Rural Development.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

IMPACT OF WEATHER PARAMETERS AND THE FUNGICIDE SPRAYING PROGRAM ON THE LATE BLIGHT (*Phytophthora infestans*) OF POTATO IN BRASOV AREA

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Abstract

Diseases like potato late blight (Phytophthora infestans) are among the major constraints that limit potato production.Climate changes have a strong impact both on the destructive potential of diseases and on the environment. A field investigation was conducted during 2023-2024 to National Institute of Research and Development for Potato and Sugar Beet Brasov. Twelve potato Romanian varieties and a fungicidal program were used. Data on first disease symptom appearance, disease incidence, and disease progress rate were recorded. The impact on the obtained yield was also analyzed. Generally, late blight severity in 2024 was greater than it was in 2023 as several varieties (Darilena and Marvis) reached high severity in both years. Furthermore, to other varieties late blight was absent (Cosiana and Asinaria in 2023) or its severity was less than 1% for the entire duration of the epidemic (Sarmis in 2023 and Asinaria in 2024).

Key words*: assessment, climatic conditions, late blight, potato, yield.*

INTRODUCTION

The oomycete *Phytophthora infestans* (Mont.) de Bary, the causal agent of the late blight disease in potato, is considered to be the most harmful pathogen in potato crops due to its rapid evolution, high adaptability and short epidemic cycle (Leesutthiphonchai et al., 2018; González-Jiméneze et al., 2023). Potato late blight was one of the main factors involved in the infamous famine in Ireland in the mid nineteenth century (Yoshida et al., 2013).

Moreover, this disease reappears in the form of different genotypes and causes huge losses in potato yields (Sawicka,and Kapsa, 2001; Yuen, 2021). Its harmfulness consists in destroying the aerial parts of plants, which in turn results in a reduction in the assimilation area and, thus, the quantity and quality of the yield of progeny tubers. The development of the disease is closely related to the meteorological conditions in potato field (Sawicka et al., 2022). It is well known that potato can be cultivated in diverse climatic conditions, and its production can be elevated significantly. Potato can yield 35 t/ ha depending on environmental conditions and the cultivated varieties (Reddy et al., 2018).

Being considered a drought-sensitive crop, potato yield is impacted by the vegetative development of pant, such as plant height, haulms number, and leaves number and size (Deblonde et al., 2001). Besides vegetative growth, drought may affect the reproductive stage of potatoes by shortening the growth cycle (Kumar et al., 2007) or by reducing the size and the number of tubers (Eiasu et al., 2007; Nasir et al., 2022).

A study conducted in Finland (Kaukaranta, 1996) showed that an increase in temperature of even one degree will lead to the appearance of late blight 4-7 days earlier and a longer time of 10-20 days in which the crop will be susceptible. Milder winters allow tubers discarded in what constitute waste pile to survive as hosts for pathogens, including *Phytophthora infestans*, and to become a source of infection when tuber debris germinates (Haverkort & Verhagen, 2008).

Also the pathogen might occur earlier in the season due to additional sources of inoculum in the soil - e.g. infected tubers from volunteer potato plants and/or oospores surviving in soil at low temperature (Quiroz et al., 2018).

Pesticide use is predicted to be higher if disease pressure increases, which will consequently affect the income from higher yields.

MATERIALS AND METHODS

The field trials were established in 2023 and 2024 to the National Institute of Research and Development for Potato and Sugar Beet Brasov. The experiment was laid out in a randomized complete block design with four replications. The experimental plot size was 18 m^2 planted with 4 rows spaced 0.75 m to each other and 0.30 m plant to plant spacing in a row.
Twelve potato cultivars were included in the trials. Their tolerance/resistance to late blight on foliage and on tuber according to registration is presented in Table 1.

Table 1. Late blight tolerance/resistant registered level (according to the NIRDPSB Brasov varieties catalogue)

Variety	Foliage resistance	Tuber resistance
Sevastia	medium resistance	medium resistance
Marvis	medium resistance	medium resistance
Castrum	medium resistance	medium resistance
Asinaria	medium resistance	medium resistance
Sarmis	medium resistance	medium resistance
Brașovia	medium susceptible	medium susceptible
Cezarina	relatively resistant	relatively resistant
Cosiana	susceptible	susceptible
Azaria	relatively resistant	relatively resistant
Ervant	medium resistance	medium resistance
Darilena	medium susceptible	medium resistance
Foresta	relatively resistant	relatively resistant

Late blight observations were performed from the first disease appearance (2023, July 3rd, and 2024, June 4th). Plots were assessed for the extent of blight spots on the leaves. Each plot was assessed as a whole for percentage disease severity using a standard international accepted severity key (Anonymous, 1947; Cruiskshank et al., 1982), as follows: 1: none or very few lesions on the leaflets (0% foliage affected), 2: (3% foliage affected), 3: (10% foliage affected), 4: (25% foliage affected), 5: (50% foliage affected), 6: (more than 50% but less than 75% stem and foliage affected), 7: (more than 75% but less than 90% affected), 8: only very few green areas of stem and leaf (much less 10%) and 9: 100% foliage destroyed.
In all cases, cultivation and maintenace was in line with current good agricultural practice.
The fungicide programs including the following fungicides applied (the active substance, and the label dosage are indicated in brackets):
2023 – Polyram DF (metiram 1.8 kg/ha), Zorvec Endavia (oxathiapripolin+benthiavalicarb 0.4 l/ha), Lieto (cymoxanil + zoxamide 0.45 kg/ha), Zorvec Endavia (oxathiapripolin + benthiavalicarb 0.4 l/ha), Carial Star (mandipropamid + difenoconazol 0.6 l/ha), Banjo (fluazinam 0.4 l/ha), and Banjo (fluazinam 0.4 l/ha).
2024 - Zetanil (cimoxanil+zoxamide 0.45 kg/ha), Cymbal 45 (cimoxanil 0.25 kg/ha), Zorvec Endavia (oxathiapripolin + benthiavalicarb 0.4 l/ha), Carial Star (mandipropamid+difenoconazol 0.6 l/ha), Cymbal 45 (cimoxanil 0.25 kg/ha), Banjo (fluazinam 0.4 l/ha), and Banjo (fluazinam 0.4 l/ha).
Yield assessment: two rows in the center of each plot were harvested mentioned the number and the weight of tubers with blight (blighted tubers assessments are usually based on a sample of 100 tubers per plot).

RESULTS AND DISCUSSIONS

In 2023 growing season was warm but no so dry. The temperatures between May and August (18.2ºC) was higher on average by 1.8ºC, compared to MAA, registering higher values by 1.1°C in June, 2.4°C in July and 3.4°C in August compared to MAA. The rainfalls in June (111.1 mm) and August (77.5 mm) exceeded the MAA (96.7 mm, respectively, 76.4 mm) as volume, but it must be stated that there were quantitatively significant rains in a short period of time After a wet August a significant precipitation deficit was registred in September, only 11 mm compared to 65.6 mm.

In 2024, in May, the air temperature was at the level of the MAA, but the amount of precipitation was much lower than the MAA values, registering only 42.7 mm compared to 82 mm. The summer months (June-August) recorded higher temperatures than the MAA, with an average of 4.6°C higher in June. Also, the volume of precipitation was much lower in all three months, establishing the phenomena of atmospheric and pedological drought. It should be emphasized that a large volume of precipitation fell in a very short time (for example, the month of August when 90% of the total quantity of the month fell in four days). The month of September recorded a temperature 3.5°C higher than the MAA and a volume of precipitation of 57.8 mm (Figures 1-2).

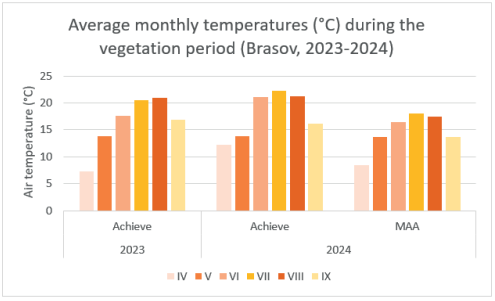


Figure 1. Air temperature during the experiment (Brasov, 2023-2024)

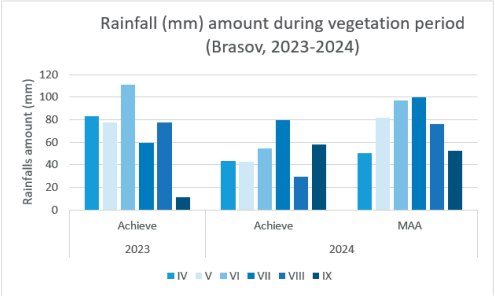


Figure 2. Rainfalls during the experiment (Brasov, 2023-2024)

In 2023, the first symptoms of the disease appear in July 3rd as a result of heavy rainfall in June and the high temperatures, but during July the situation had changed (Figure 3). There was a low volume of precipitation, with 40.7 l/mp less than MAA accompanied by a temperature level higher with 2.4°C, and the disease ability to manifest has been diminished.

In 2024, the first symptoms of late blight were observed on June 4 to the Cosiana variety (Figure 4). However, it must be emphasized that the attack was of low intensity due to the climatic conditions, severe drought, lack of precipitation over long periods of time. The preventive application of treatments also contributed to the inactivation of the fungus, the possibilities of sporulation being very low.

Currently, European legislation requires member states to promote a disease and pest control policy that uses fewer chemicals within an integrated plant protection management. Knowing the mode of action of fungicides and using them according to the situation in the field contributes to reducing losses.

Carrying out the registration and the subsequent analysis of climatic variables is fundamental to the development of agronomic and sanitary management recommendations for the crop (Kessel et al., 2018). Climatic variables such as relative humidity, precipitation, and temperature are relevant factors for the proper development of late blight epidemic (Rodríguez et al., 2023).

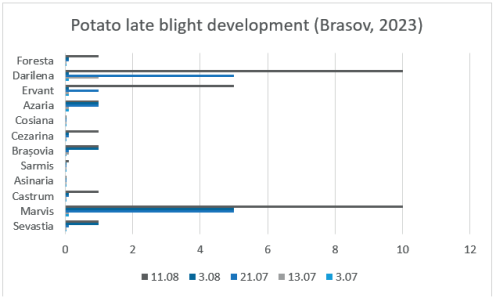


Figure 3. Potato blight development dynamics in potato cultivars (Brasov, 2023)

In 2023, first signs of late blight presented varieties Marvis, Azaria, Ervant, Darilena in 3rd July, when the first treatment (Polyram DF) was done after ten days, at the time of te second treatment (Zorvec Endavia) also Brasovia variety presented symptoms. Disease was found only on the leaves. However, the infection rate was very low at approximately 0.1-0.5% of total foliage surface. Two weeks later, on the 21st of July, late blight spots were noticed on foliage of the varieties Marvis and

Darilena, but the disease developed very slowly, still progressing till 11 of August.

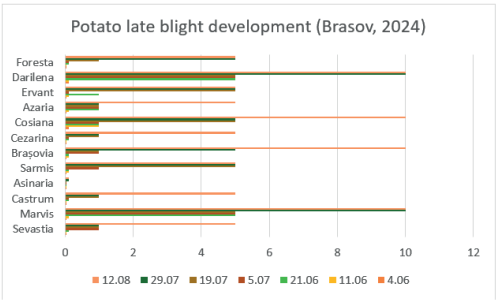


Figure 4. Potato blight development dynamics in potato cultivars (Brasov, 2024)

At the time of the first treatment (Zetanil), some spots were observed only in the Cosiana and Darilena varieties. Assessments were made and sporadic spots were recorded in most varieties and was applied the second treatment (Cymbal 45). Starting with the third treatment (Zorvec Endavia), a differentiation is produced based on the climatic conditions and the resistance level of the variety in the given specific conditions. Thus, after the fourth treatment (Carial Star), the Asinaria variety responded best, followed by the Cezarina, Ervant and Castrum. At the end of the assessments, the intensity of the attack was between 0.1% to Asinaria variety and 10% to Marvis, Brasovia, Cosiana and Darilena.

Prophylactic spray with contact fungicide and second spraying with translaminar/ contact fungicide when the first symptoms appeared, followed by third spraying with systemic or contact fungicide according to the climatic conditions and the two final spraying with contact fungicide effectively managed the late blight disease.

The weather was not conducive for late blight, the disease development was impeded in both years, because of high temperature and lack of rainfalls.

We subscribe to what other authors have observed (Cucak et al., 2021), namely that potato production is completely dependent on the prophylactic use of fungicides due to the high potential risks of crop loss associated with potato late blight outbreaks.

Significant differences in yields between varieties were observed in September 2023.The medium late variety Cosiana had a lower yield (21.11 t/ha), which can be justified by the later maturity, but Foresta (14.0 t/ha) and Darilena (21.06 t/ha) did not give the expected results. In terms of total yield, Ervant (45.47 t/ha), Castrum (41.31 t/ha), and Azaria (40.69 t/ha) varieties were homogeneous, whereas for marketable yield, the Marvis (33.36 t/ha) and Asinaria (31.11 t/ha) varieties were homogeneous in this trait. Sarmis being a cultivar that accumulates early, the production of large tubers was high (11,92 t/ha), the total yield (26,94 t/ha) being similar with that of Cezarina (26,08 t/ha) and Sevastia (25,69 t/ha) varieties, which, however had a high production of tubers of 35-45 mm (the seed fraction). Also Brasovia variety was registered as having a high seed production (20.72 t/ha) from the total of 26.11 t/ha) (Table 2).

The visual inspection made at the harvest not revealed latently infected tubers. Tubers with low infection severity may be overlooked and therefore, after storage for the seed material in the following year, in spring is made another selection.

Table 2. Potato yield obtain in 2023 to NIRDPSB Brasov

Variety	Tuber >45 mm yield	Tuber 35-45 mm yield	Tuber <35 mm yield	Total yield
Cosiana	8,03	11,33	1,75	21,11
Marvis	12,28	21,08	1,69	35,06
Darilena	6,19	14,17	0,69	21,06
Asinaria	13,08	18,03	0,53	31,64
Sarmis	11,92	13,94	1,08	26,94
Cezarina	7,53	16,78	1,78	26,08
Foresta	6,03	7,19	0,78	14,00
Ervant	25,03	19,31	1,14	45,47
Sevastia	7,14	17,69	0,86	25,69
Azaria	20,50	19,19	1,00	40,69
Castrum	16,03	23,36	1,92	41,31
Brașovia (Mt)	4,03	20,72	1,36	26,11
DL 5%	3,78	3,66	0,84	4,99
DL 1%	5,06	4,91	1,13	6,69
DL 0.1%	6,68	6,48	1,49	8,83

In 2024, the lack of water and excessive temperatures lead to the installation of the phenomenon of excessive drought, with serious consequences on potato production.

At the harvest, on September 19, it was observed that Ervant and Azaria varieties recorded the highest number of large (5.22 t/ha, respectively 4.86 t/ha) tubers, differentiating very significantly from the other varieties. Also, Azaria (16.97 t/ha), Ervant (16.94 t/ha) and Castrum (16.58 t/ha) registered a significant number of tubers in the 35-55 mm fraction, which actually represent the seed potato. However, the Darilena variety

registered a very significant number of small tubers, which is not gratifying.
The total production was not at the level of the productive potential of any of the varieties. The recorded values were between 25.72 t/ha for the Azaria variety and 7.58 t/ha for the Cezarina variety (Table 3).

Table 3. Potato yield obtain in 2024 to NIRDPSB Brasov

Variety	Tuber >45 mm yield	Tuber 35-45 mm yield	Tuber <35 mm yield	Total yield
Cosiana	1,81	14,50	4,31	20,61
Marvis	1,89	7,44	2,69	12,03
Darilena	0,75	7,03	6,50	14,28
Asinaria	2,67	11,75	2,97	17,39
Sarmis	1,58	9,14	3,39	14,11
Cezarina	0,67	4,64	2,28	7,58
Foresta	2,50	9,00	1,53	14,00
Ervant	5,22	16,94	2,53	24,69
Sevastia	3,19	8,69	1,19	13,08
Azaria	4,86	16,97	3,89	25,72
Castrum	2,08	16,58	3,19	21,86
Brașovia (Mt)	0,53	8,56	3,50	15,58
DL 5%	2,18	3,41	1,27	3,34
DL 1%	2,92	4,57	1,70	4,47
DL 0.1%	3,85	6,03	2,24	5,90

Comparing the productions obtained in 2024 with those of 2023, which from a climatic point of view was warmer and with less precipitation, was observed that most varieties (Castrum, Azaria, Ervant, Sevastia, Asinaria, Sarmis and Brașovia) had production halved, Marvis and Cezarina registered dramatic decreases, Cosiana and Foresta varieties maintained their production at low level.

CONCLUSIONS

There is already an observed warming trend and major changes in the distribution of precipitation over an agricultural year. Currently are in use a large number of late blight forecasting modes which gives satisfactory information. Thus, the implementation of a forecasting model reduces the frequency of fungicides application up to 50 % as compared with conventional, calendar based schedule.
Chemical control must be judicious and treatments carried out only when necessary to keep disease below economic thresholds. A spray program that alternates between fungicides may be effective in controlling the disease but also in avoiding the build-up of resistance of *Phytophthora infestans*.
In the years 2023-2024 at NIRDPSB Brasov in adverse climatic conditions, high temperatures, the lack of precipitation and at the time of their appearance, their unevenness, made the potato late blight not manifest at an epidemic level. By also applying foliar treatments at "key moments", the control of the disease was effective.

ACKNOWLEDGEMENTS

This research work was carried out with the support of Ministry of Research, Innovation and Digitalisation and was financed from Project PN No. 40N/2023 (PN 23-19-02-02).

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

STUDY REGARDING THE INFLUENCE OF NITRIC, AMMONIACAL NITROGEN FERTILIZATION AND VARIETY ON WINTER WHEAT (*Triticum aestivum* L.) PROTEIN CONTENT

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Abstract

The aim of this study was to analyse e the effect of variety, nitrogen rate and type and their interaction on protein content and to determine the economic feasibility of varieties and fertilizer application for the highest values. The subject of the experiment consisted in testing during two wheat growing seasons 2021-2023, twenty-seven winter wheat varieties fertilized with nitric and ammoniacal nitrogen in three different rates - 120, 150 and 170 kg N ha^{-1}. The layout of the experimental plan was done using stratified randomization block method. Cultivars were factorially combined and arranged in completely randomized blocks. We concluded that compared with the mean of the experience of 14.75%, variety and nitrogen fertilization systems interaction had a significant effect on wheat protein content. The highest protein content was obtained by the Tika Taka variety - 15.78%. Regarding the fertilization system, with very significant differences the values above the average of the experience, 15.32%, 15.27% and 15.36% were obtained the levels of fertilization with nitric nitrogen and those with ammoniacal nitrogen had negative values of 14.04%, 14.20% and 14.32%.

Key words*: ammoniacal N, nitric N, fertilization rate, protein content, wheat genotype.*

INTRODUCTION

Wheat is the second most widespread cultivated plant in the world, it is estimated that this crop covers 200 million hectares, able to grow in temperate, mediterranean and subtropical regions of the two hemispheres, thanks to its enormous genetic diversity with more than 25,000 varieties of *Triticum aestivum* L. adapted to different climate and soil conditions (Frankin et al., 2021; de Sousa et al., 2021). Due to relatively modest cultivation requirements, high adaptability to climate and soil, and high nutritional value, wheat spread rapidly from its center of origin, a small area in the Middle East (Jordan, Palestine, and Lebanon) to Syria, Turkey, Iraq and Iran and then around the world, propelling itself into the orbit of eternity thanks to its importance (Harari, 2015).

Nitrogen is an important factor limiting plant growth and consequently wheat pro-duction and quality worldwide. Plants consume nitrogen present in both the atmospheric air and soil minerals. The ability of plants to take up nitrogen naturally or applied as fertilizers is one of the critical factors limiting the efficient use of N by plants. Despite the fact that N is one of the most abundant elements on earth, nitrogen deficiency is one of the most common problems affecting wheat plant growth and development (Rossini et al., 2018; Noor et al., 2023).

Wheat generally contains 3-5% nitrogen in its tissue biomass, which is by far the most important soil-derived nutrient outside of oxygen, hydrogen, and carbon (Ali et al., 2011). Nitrogen has a complex role, it is responsible for plant growth and development, involved in a wide range of physiological processes such as photosynthesis, protein synthesis and enzyme activity, it is a constituent of nucleic acids (DNA and RNA), proteins, enzymes, cell wall and pigment system, amino acids, vitamins (biotin, thiamin, niacin and riboflavin), all proteins and a wide range of nitrogen-containing organic molecules. Nitrogen assimilation occurs

throughout the growing season, but with different intensities depending on the growth and development phenophase and critical periods of nutrition (Ali et al., 2011; Barraclough et al., 2014).

Nitrogen is indispensable for both production yields and wheat quality. The benefits of nitrogen fertilizer on yield and quality depend on the rate and timing of application (Grzebisz et al., 2023; Bogard et al., 2010).

The use of mineral fertilizers in agricultural systems for growing wheat is essential to support the growing world population. Wheat is demanding in terms of fertilization, be-cause the root system is poorly developed, given the architecture of the root, therefore it explores a small volume of soil and has a low power to solubilize and absorb nutrients from the soil reserve (Ma et al., 2021).

Nitrogen is the essential element for increasing production yields and economic profitability. Nitrogen availability for wheat production is almost as important as soil water availability. Without an adequate N supply, all growth stages are severely stunted (Zhong et al., 2019; Song et al., 2020). N applied at sowing stimulates tillering and vegetative growth, while N applied in the generative phases has a greater influence on the protein concentration of the caryopses (Habash et al., 2006). Studies suggest that N use efficiency is higher when N is applied later (at tillering) than when applied at sowing (Zhong et al., 2018). Doses, types of nitrogen fertilizers and the time of N application influence nitrogen uptake and its translocation within the plant (Khan et al., 2022). Thus, the timing of fertilizer application and doses have a significant role in determining the amount of absorption and its efficient use by the plant. N from the above-ground parts of plants is actively recycled and transported into plants as they grow. This redistribution of nitrogen from aboveground parts of wheat has been studied extensively. The flow and amount of nitrogen redistributed to developing caryopses varies according to the availability-absorption ratio, which is influenced by climatic conditions (temperature, precipitation) and inherent properties of plant organs (Ahmed et al., 2020; Guo et al., 2019). It has also been suggested that N remobilization from roots may play an important role in whole plant N utilization (Daba et al., 2021).

Increased leaf mass improvement in wheat plants when nitrate nitrogen fertilizers are applied compared to ammoniacal nitrogen fertilizers (Aluko et al., 2023). Growth factors consisting of tiller number, root volume, specific leaf mass, standard leaf area, and total leaf area per-formed better with NO_3^- compared to NH_4^+ fertilizers. Along with the above, the use of nitrogen fertilizers based on ammoniacal nitrogen decreases the nitrate content of plants, while nitrogen fertilizers based on nitric nitrogen increase it (He et al., 2022). Furthermore, a significant decrease in the soluble sugar content of wheat caryopsis exposed to NH_4^+ compared to NO_3^- was observed (Sinha et al., 2020). In addition, investigations indicated higher plant nitrogen use efficiency in wheat plants fertilized with NO_3^-, as well as reduced photosynthesis and carbon assimilation rates in the case of NH_4^+ fertilization (Ukalska-Iaruga et al., 2020; Yadav et al., 2023).

Today we share in the success of agriculture's evolution - a two-sided coin, given the ever-growing population and the climate crisis. From 1.6 billion in 1900, the world's population has grown to more than 7 billion today and will reach 9 billion by 2050. In this context, it will be impossible to feed the ever-growing population without a significant increase in wheat quality. Data from the United Nations show us that in the next 30 years the global demand for food will increase by 70% as a result of population growth, and the battle of the future will be over food and water. Food security is systematically analyzed by the Food and Agriculture Organization of the United Nations, which draws attention to the anticipated food crisis. Thus, international forums explain why population growth is likely to outpace production yields and call for greater investment to keep pace with future demand. According to a 2022 Food and Agriculture Organization (FAO) report, global wheat production was 777 million tons, production that needs to be doubled by 2050 and tripled by 2100 to ensure food security, as a result of population growth. How this will be achieved is unknown, as the extent of cultivated arable land is not expected to

increase, but rather to decrease as a result of urbanization, increased soil erosion, and areas affected by drought and salinization (UNESCO; FAO 2024; European Commission; From Farm to Fork; Mălinaș et al., 2022).

MATERIALS AND METHODS

The study was conducted in 2021-2023 in the Dudeștii Noi experimental fields (45°50′51″N 21°06′30″E, 87 m altitude, Garmin device GPSMAP 64 st), located in the Western Plain of Romania, which is a part of one of the largest agricultural regions of the country. The territory of the area where the research was carried out is located in the forest-steppe area, with a high hydrostatic level of the water table and differentiated parental rocks, which determined a varied pedological background. The formation of sedimentary rocks in this area is closely related to the existence of the Pannonian Lake, which, by retreating, created an area of accumulation of coarse materials brought to the unstable bed of the Mureș River. The parent rocks, on the basis of which the soils around the commune of Dudeștii Noi were formed, consist of quartz sands with a varied content of calcium carbonate, as well as medium-textured löessoid materials. In some places, in micro-depression zones, the water table has salted the mentioned rocks, thus forming areas with salinized or alkalized soils. The experimental field was located on a cambic chernozem soil, wet phreatic, slightly levigated, medium clay loam, developed on fluvial, carbonate, medium fine materials with a well-defined profile and with insignificant differences regarding the physical, hydric and chemical properties. The chernozems in general and the cambium equally represent soils with a bioenergetic potential and a good production capacity. Cambic chernozem is a very deep soil with loamy texture, generated by transported and redeposited materials, constituted by aeolian deposits (loess and carbonate loessoid deposits) (Crista, 2017). The soil profile is characterized by the sequence of the following horizons: Ap, Am, Bv, Ck. Ap horizon: 0-15 cm, gradual transition, dark gray brown clayey texture, small glomerular structure, plastic, weak adhesive, small pores, frequent roots, dry. Am horizon: 15-35 cm, gradual transition, dark brown loamy texture, medium glomerular structure, well developed, plastic, adhesive, small pores, weak compact, thin roots frequent. Bv horizon: 35-60 cm, gradual transition, dark gray-brown loamy texture, medium subangular polyhedral structure, plastic, adhesive, fine porous, moderately compact, sparse thin roots, dry. Ck horizon: 60-100 cm, gradual transition, yellowish brown light gray loamy texture, subangular and medium angular polyhedral structure well developed, plastic, adhesive, dry (Munteanu, 2012).

Physical and hydraulic properties: The loamy-clay texture - dusty in the first 70 cm and loamy-dusty in the rest, makes the apparent density (AD) medium throughout the profile of the soil, with 1.46 g/cm^3 in the Ap horizon and 1.58 g/cm^3 in Am. Total porosity (TP) is medium in Ap (48.8%) and high in Am (58.2%). Air porosity (AP) is medium in Ap (15.2%), high in Am (23.8%), medium in Bv (21.7%) and low in Ck (12.2%). The ranges of variation for clay are between 33.4-40.3%. The processed Ap horizon has a clay content of 36% compared to the Am horizon with a content of 40.3%. In horizons Bv and Ck, the amount of clay shows lower values (33.8%) and 33.4%, respectively. The soil is weakly to moderately compacted on the horizons below the tilled horizon. The values of the hydrophysical indices show different values, correlated with the physical properties. A medium value of the withering coefficient is noted in the Ap and Am horizons (11.7%), high in Bv (13.2%) and medium in Ck (12%) (Obrejanu, 1964).

The field capacity (FC) is medium over the entire soil profile, with values of 23.3% in Ap, Am, Bv and 22.4% in Ck. Chemical properties were determined using the Kjeldahl and Olsen et al. methods (Dumitru et al., 2011). The soil has a humus content of 3.04% in the Ap horizon and 1.78% in the Am horizon, decreasing by 1.24% in the Bv horizon. It is medium supplied in total nitrogen, with 0.172% in Ap, very well supplied in Am with mobile phosphorus (168 ppm) and well supplied with mobile potassium (248 ppm). The pH was tested using the distilled glass electrode method, 1:2.5 soil and water. The reaction of the soil on the profile is weakly acid in Ap horizon 5.58, weakly alkaline 7.66. The amount

of exchangeable bases is medium (23.2 in Ap, 24.8 in Am), and the degree of saturation in bases shows values of 92.62% which gradually increases on the Bv horizon. Trace element content is high for zinc (176 ppm) in the Ap and extremely low in the Bv horizon (0.40 ppm). Climatic data during the period of the experiment were collected from the meteorological station of Dudeștii Noi and are represented in Figure 1. The climate of this area is temperate - continental with slight Mediterranean influences. The average annual temperature is 10.8°C and the warmest month was July. Temperatures were also extremely high for September and October. As can be seen, during the entire experimental period and especially during the vegetation months, the average monthly temperatures exceeded the multiannual averages, with the highest values during the vegetation period being recorded between June and September in all two experimental years.

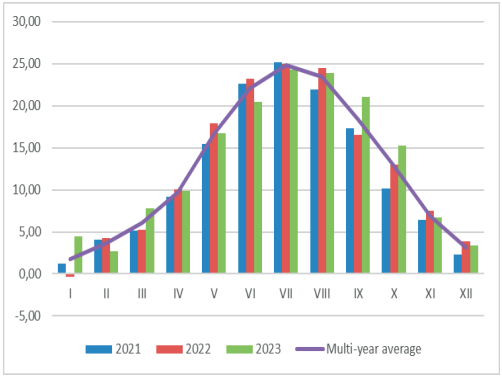


Figure 1. The average monthly temperatures (°C) recorded in Dudeștii Noi (2021-2023)

The average annual precipitation was frequently around 540 mm. In May and July, as a rule, the maximum pluviometric occurs (Figure 2). Sowing was carried out late, after successful application of a watering to help the suitable preparation of the field. The months from August until October were dry. These were registered against the back-ground of acute lack of precipitation. The last three years, especially, have positioned the western area of Romania in an unprecedented position. The Western Plain of Romania, an important region of the country for wheat cultivation, has recently had to deal with long periods of very hot and dry weather and very wet and rainy periods with heavy rainfall in a short period of time. These climate events often exceed the relevant meteorological observations, some of which vary strongly from the multi-year mean values recorded to date.

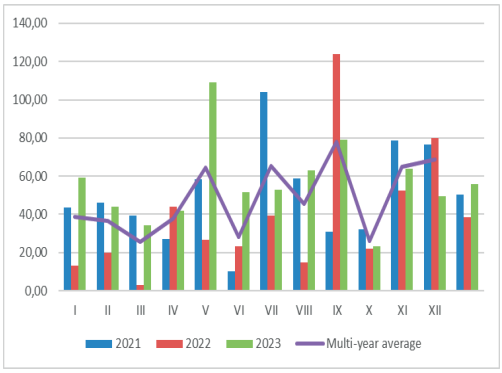


Figure 2. The average monthly rainfall (mm) recorded in Dudeștii Noi (2021-2023)

The biological material is represented by 27 Romanian, German and French autumn wheat varieties, some of the most cultivated wheat varieties in the Western Plain of Romania, and the criteria that were the basis of their choice are the high production potential, high tolerance to disease and pests attack and specific qualities for milling and baking indices.

The layout of the experimental plan was made using the stratified randomization block method. Cultivars were factorially combined and arranged in completely randomized blocks. This experimental method was chosen in order to avoid the interfering effects of various environmental factors and to adequately and accurately estimate nitrogen utilization. The size of each plot was 1.2 m × 7 m, the distance between plots and adjacent blocks was kept at 0.5 m and 1.5 m, respectively, and the buffer strip was 2.5 m. Each block consisted of 27 plots with three replicates. The study is based on a trifactorial experiment, in subdivided plots, on the 27 × 3 type, with the following grading of the experimental factors:

FACTOR A - cultivated wheat variety:

a1 - Dacic, a2 - Miranda, a3 - Alex, a4 - Litera, a5 - Ciprian, a6 - Crișana, a7 - Biharia, a8 - Glosa, a9 - Bohemia, a10 - Sothys, a11 - Sacramento, a12 - Rubisko, a13 - Certiva, a14 -

Aurelius, a15 - Aspect, a16 - Papillon, a17 - Activus, a18 - Centurion, a19 - Tika Taka, a20 - Chevignon, a21 - Sosthene, a22 - Vivendo, a23 - Sophie, a24 - Solindo, a25 - Tiberius, a26 - Arrezo and a27 - Apexus.

FACTOR B - fertilization system:

b1 - Ammoniacal nitrogen; b2 - Nitric nitrogen.

FACTOR C - fertilization level:

c1 - 120 kg ha^{-1} N a.s.; c2 - 150 kg ha^{-1} N a.s.; c3 - 170 kg ha^{-1} N a.s.

Nitrogen doses were established starting from the maximum ceiling accepted by The Agency for Intervention and Payments in Agriculture in Romania, 170 kg N ha^{-1} (APIA, 2021). The lowest dose of 120 ha^{-1} was chosen according to the use of nitrogen in wheat crop, the humus content and the available nitrogen in the soil. The fertilizer consisted of two types of nitrogen (nitric and ammoniacal) that were applied in the form of ammonium sulfate $(NH_4)_2SO_4$ and calcium nitrate $Ca(NO_3)_2$, in three contrasting fertilization doses and administered to the plants in - a single dose, unfractionated, at the stage of growth BBCH 30-31. In both years of study, the sowing rate for each variety was established considering aspects related to the sowing rate such as purity, germination and the 1,000 kernel weight, and the density at sowing was 550 germinating grains/m^2. Wheat was sown at a depth of 4 centimeters with a row spacing of 12.5 cm. The treatments applied were V1 - nitric N dose of 120 kg ha^{-1} N a.s., V2 - nitric N dose of 150 kg ha^{-1} N a.s, V3 - nitric N dose of 170 kg ha^{-1} N a.s, V4 - ammoniacal N dose of 120 kg ha^{-1} N a.s., V5 - ammoniacal N dose of 150 kg ha^{-1} N a.s, V6 - ammoniacal N dose of 170 kg ha^{-1} N a.s. and the Mt - control variant was the average of the experimental field.

The wheat samples were cleaned in a fully automated process using the MLN Sample Cleaner - Pfeuffer equipped with a cyclone for light bodies, a ball screening system for cleaning, additional screening screens for sorting coarse and small grains and then homogenized with an automatic grain sampler Vario 1G - Pfeuffer equipped with an integrated electric actuator (adjustment cylinder) according to ISO 24333:2010 (ASRO, 2022), then the tests for bakery quality indices were carried out.

Protein content was measured using a whole seed NIR multiparameter analyzer using near-infrared transmission technology (transmittance). A volume of 600 milliliters of sample was used to analyze this parameter. The measurement process was automated, the transport rotor ensured constant density, and the built-in infrared spectrometer with a range of 950 to 1,540 nanometers scanned each sample 1,500 times. Thanks to this technology, each sample was completed by printing an analysis report in less than a minute. In parallel, two determinations were performed for each analyzed sample.

Based on the results of two years field experiment, the wheat protein content parameter was calculated using the Analysis of variance (ANOVA) and Student t tests.

RESULTS AND DISCUSSIONS

Wheat quality is a complex trait resulting from interactions between numerous protein components. Protein substances usually represent 10-16% of the mass of the grain (with limits between 8 and 24%) and are mostly located towards the peripheral parts of the grain (coats, aleurone layer), in the embryo and scutellum. The amount and composition of proteins give the nutritive quality of the grain. Proteins form, mainly, gluten, a mixture of protein substances that occupy the space between the starch granules in the endosperm and which, after grinding, in the flour, embeds the starch granules. The accumulation of proteins in the grain depends on a number of factors, such as: the wheat species, the variety, the climatic conditions, the natural fertility of the soil and the doses of nitrogen fertilizers used. Wheat grains contain on average 8-30% proteins, which are divided into two major categories: prolamins which include gliadins and glutenins, and nonprolamins consisting of water-soluble albumins and saline-soluble globulins. The albumin/globulin fraction accounts for 20-25%, while gliadins and glutenins account for 75-90% of wheat proteins (Sulek et al., 2018; Liu et al., 2022). Wheat storage proteins, namely gliadins and glutenins, are the main components of gluten, which determine the nutritional values and baking properties of wheat grains due to their impact

on the water absorption capacity of the dough, its elasticity and extensibility. Gliadins give the dough extensibility and viscosity and glutenins are responsible for elasticity and resistance (Lafiandra et al., 2022; Veraverbeke et al., 2002).

Table 1. Variation analysis

Variation source	SSP (SP)	Degree of Freedom	Weighed Least of Squares - WSL (s^2)	F test for s^2 error		
				Value	P	Signification
A (variety)	137.63	26	5.29	6.5	0.000000	***
B (agrofund)	159.26	5	31.85	39.3	0.000000	***
A×B	102.98	130	0.79	1.0	0.551313	ns
Error	262.39	324	0.81			
Total	662.26					

*, **, or *** indicate statistically significant differences between sample means and Mt based on t-test, at $p \leq 0.05$, $p \leq 0.01$, or $p \leq 0.001$, respectively. NS (not significant) indicates the t-test difference between sample means was $p > 0.05$.
0, 00 or 000 also indicate significant differences between sample means and Mt based on t-test at $p \leq 0.05$, $p \leq 0.01$, or $p \leq 0.001$, respectively, but with negative values.

The F test (Table 1, column p) shows that: factor A - variety and factor B - agrofund had a very significant effect, that is: between the varieties followed in the experiment there are very significant differences; between the 6 agrofunds there are very significant differences; the interaction A × B, insignificant effect, the 27 varieties did not react differently within the 6 agrofunds. In conclusion, the null hypothesis H0 is rejected for factor A (variety) and factor B (agrofund), it is accepted for the interaction A × B.

Table 2. Student test for factor A (variety) – witness (Mt), average of the field

Variety	Protein content (%)	Difference (%)	Signification
a1 - Dacic	14.98	0.22	ns
a2 - Miranda	14.41	-0.34	ns
a3 - Alex	14.84	0.09	ns
a4 - Litera	14.87	0.12	ns
a5 - Ciprian	15.68	0.92	**
a6 - Crișana	15.05	0.30	ns
a7 - Biharia	15.03	0.28	ns
a8 - Glossa	14.35	-0.40	ns
a9 - Boema	14.79	0.04	ns
a10 - Sothys	14.18	-0.57	ns
a11 - Sacramento	13.95	-0.80	00
a12 - Rubisko	14.08	-0.68	0
a13 - Certiva	14.23	-0.52	ns
a14 - Aurelius	15.32	0.57	ns
a15 - Aspekt	14.68	-0.08	ns
a16 - Papillon	13.99	-0.76	*
a17 - Activus	14.85	0.10	ns
a18 - Centurion	13.75	-1.00	000
a19 - Tika Taka	15.78	1.03	***
a20 - Chevignon	15.34	0.59	*
a21 - Sosthene	14.30	-0.45	ns
a22 - Vivendo	14.89	0.14	ns
a23 - Sophie	15.02	0.27	ns
a24 - Solindo	14.57	-0.18	ns
a25 - Tiberius	15.69	0.94	**
a26 - Arrezo	14.95	0.20	ns
a27 - Apexus	14.71	-0.04	ns
Mean	14.75	Mt	
DL 5% = 0.584; DL 1% = 0.769; DL 0.1% = 0.981			

*, **, or *** indicate statistically significant differences between sample means and Mt based on t-test, at $p \leq 0.05$, $p \leq 0.01$, or $p \leq 0.001$, respectively. NS (not significant) indicates the t-test difference between sample means was $p > 0.05$.
0, 00 or 000 also indicate significant differences between sample means and Mt based on t-test at $p \leq 0.05$, $p \leq 0.01$, or $p \leq 0.001$, respectively, but with negative values.

The protein content achieved using the 27 winter wheat varieties is shown in Table 2. Compared to the control - the average of the experience of the two years of study, significant differences were recorded by the Papillon and Chevignon varieties; distinctly significant differences were recorded by the Ciprian and Tiberius varieties, and very significant values were recorded by the Tika Taka variety. Below the experience average were the Rubisko varieties with significant differences, Sacramento with distinctly significant differences and Centurion with very significant differences. The other varieties did not register differences. The variety with the highest value of this index is Tika Taka - 15.78%, Tiberius - 15.69% and Ciprian - 15.68%. The varieties Sacramento - 13.95% and Centurion - 13.75% ranked below the experience average. The other varieties did not register differences.

Table 3. Student test for factor B (level of fertilizer) – witness (Mt), average of the field

Variant	Protein content (%)	Difference (%)	Signification
V1 - 120 kg ha^{-1} N a.s nitric N	15.32	0.57	***
V2 - 150 kg ha^{-1} N a.s nitric N	15.27	0.52	***
V3 - 170 kg ha^{-1} N a.s nitric N	15.36	0.61	***
V4 - 120 kg ha^{-1} N a.s ammoniacal N	14.04	-0.72	000
V5 - 150 kg ha^{-1} N a.s ammoniacal N	14.20	-0.55	000
V6 - 170 kg ha^{-1} N a.s ammoniacal N	14.32	-0.43	00
Mean	14.75	Mt	
DL 5% = 0.275;	DL 1% = 0.363;	DL 0.1% = 0.462	

*, **, or *** indicate statistically significant differences between sample means and Mt based on t-test, at $p \leq 0.05$, $p \leq 0.01$, or $p \leq 0.001$, respectively. NS (not significant) indicates the t-test difference between sample means was $p > 0.05$.
0, 00 or 000 also indicate significant differences between sample means and Mt based on t-test at $p \leq 0.05$, $p \leq 0.01$, or $p \leq 0.001$, respectively, but with negative values.

The protein content achieved using the nitric and ammoniacal nitrogen fertilization is shown in Table 3. Compared to the control group - the mean of the experience of the years of study, very significant differences were recorded, regardless of the level or type of N fertilizer.

Table 4. Student test for A × B interaction (variety × level of fertilization) – witness (Mt), average of the field

Variety	V 1		V 2		V 3		V 4		V 5		V 6	
	P	Diff	P	Diff	P	Diff	P	Diff	P	Diff	P	Diff
a1 - Dacic	15.40	0.62	15.70	0.60	15.75	0.25	14.00	0.17	14.20	0.61	15.05	0.62
a2 - Miranda	15.05	0.62	14.45	0.60	14.85	0.25	13.85	0.17	14.15	0.61	14.10	0.61
a3 - Alex	15.60	0.62	15.65	0.60	16.00	0.25	12.85	0.17	14.40	0.61	14.55	0.61
a4 - Litera	15.30	0.62	14.25	0.60	14.90	0.25	13.25	0.17	15.65	0.61	15.85	0.61
a5 - Ciprian	16.70	0.62	15.8	0.60	16.60	0.25	14.50	0.17	15.35	0.61	15.10	0.61
a6 - Crişana	16.20	0.62	15.00	0.60	15.10	0.25	15.40	0.17	14.25	0.61	14.35	0.61
a7 - Biharia	16.05	0.62	16.50	0.60	15.65	0.25	13.90	0.17	13.75	0.61	14.35	0.61
a8 - Glossa	15.20	0.62	14.80	0.60	15.05	0.25	13.35	0.17	13.75	0.61	13.95	0.61
a9 - Boema	15.15	0.62	15.25	0.60	15.40	0.25	14.20	0.17	14.40	0.61	14.35	0.61
a10 - Sothys	15.00	0.62	14.50	0.60	14.60	0.25	13.70	0.17	13.40	0.61	13.90	0.61
a11 - Sacramento	14.75	0.62	14.20	0.60	14.40	0.25	13.80	0.17	13.05	0.61	13.50	0.61
a12 - Rubisko	14.50	0.62	14.85	0.60	15.00	0.25	13.15	0.17	13.60	0.61	13.35	0.61
a13 - Certiva	14.70	0.62	14.85	0.60	14.75	0.25	13.65	0.17	13.50	0.61	13.95	0.61
a14 - Aurelius	16.40	0.62	15.90	0.60	15.70	0.25	14.65	0.17	14.65	0.61	14.60	0.61
a15 - Aspekt	14.50	0.62	16.10	0.60	15.60	0.25	14.45	0.17	13.85	0.61	13.55	0.61
a16 - Papillon	14.50	0.62	14.35	0.60	14.45	0.25	13.75	0.17	13.35	0.61	13.55	0.61
a17 - Activus	15.40	0.62	15.90	0.60	15.95	0.25	14.40	0.17	13.60	0.61	13.85	0.61
a18 - Centurion	14.55	0.62	14.00	0.60	14.15	0.25	12.85	0.17	13.60	0.61	13.35	0.61
a19 - Tika Taka	16.90	0.62	16.40	0.60	16.10	0.25	15.20	0.17	14.90	0.61	15.20	0.61
a20 - Chevignon	16.30	0.62	15.80	0.60	15.70	0.25	15.10	0.17	14.60	0.61	14.55	0.61
a21 - Sosthene	14.70	0.62	14.75	0.60	15.70	0.25	13.30	0.17	13.55	0.61	13.80	0.61
a22 - Vivendo	14.85	0.62	14.90	0.60	14.85	0.25	14.50	0.17	14.90	0.61	15.35	0.61
a23 - Sophie	13.20	0.62	16.10	0.60	16.10	0.25	15.10	0.17	15.15	0.61	14.45	0.61
a24 - Solindo	15.45	0.62	15.40	0.60	15.05	0.25	13.70	0.17	13.85	0.61	13.95	0.61
a25 - Tiberius	16.55	0.62	16.25	0.60	16.05	0.25	15.15	0.17	14.95	0.61	15.20	0.61
a26 - Arrezo	15.60	0.62	15.90	0.60	16.15	0.25	13.30	0.17	14.40	0.61	14.35	0.61
a27 - Apexus	15.05	0.62	14.85	0.60	15.10	0.25	13..90	0.17	14.65	0.61	14.70	0.61
Mean	14.75											
DL 5% = 0.486;	DL 1% = 0.528;						DL 0.1% =0.667					

*, **, or *** indicate statistically significant differences between sample means and Mt based on t-test, at $p \leq 0.05$, $p \leq 0.01$, or $p \leq 0.001$, respectively. NS (not significant) indicates the t-test difference between sample means was $p > 0.05$.
0, 00 or 000 also indicate significant differences between sample means and Mt based on t-test at $p \leq 0.05$, $p \leq 0.01$, or $p \leq 0.001$, respectively, but with negative values.

However, it should be noted that: at the V1, V2 and V3 treatments the protein content values obtained are above the experience average.
The highest value of 15.36% was obtained at the V3 treatment, followed by V2 - 15.27% and the minimum level of nitrogen fertilization, V1 - 15.32%. Below the experience mean are the protein content values from the ammoniacal nitrogen fertilization levels, the values being very significant for the first two nitrogen rates, V4 and V5 and distinctly significant for the maximum level of fertilization with this type of nitrogen.
There are no significant differences in Table 4, the same is confirmed by the F test from the analysis of variance.
Influence of variety on protein content - the protein content values range between 13.7% (Centurion variety) and 15.7% (Tika Taka variety). All other 25 varieties have values between 13.8 and 15.5%. The differences between varieties are highly significant ($p<0.001$), according to the F-test value.
Influence of fertilization system on protein content - for the first three fertilization levels, the protein content values are approximately identical. From the fertilization level of 120 kg ha^{-1} a.s. ammoniacal nitrogen to that of 170 kg ha^{-1} a.s. ammoniacal nitrogen, the trend is slightly upward, with protein content values ranging between 14 and 14.4%. The differences between agrofunds are very significant ($p<0.001$), according to the F-test value.
Influence of the A × B interaction on protein content: the lowest value of this index was recorded for the Alex variety - 12.9% at the fertilization level of 120 kg ha^{-1} a.s. ammoniacal nitrogen; and the highest value of this index was obtained by the Tika Taka variety - 16.9% at the fertilization level of 120 kg ha^{-1} a.s. nitric nitrogen.
Factor A - variety contributes to the formation of the protein content index by 20.78%, factor B - fertilization system by 24.05%, and the interaction of the two mentioned factors A × B by 15.55% (Figure 3). However, it is very important to mention that the greatest contribution to the formation of protein content is made by other factors that were not taken into account in this study, being followed by factor B - fertilization system, then with a very small difference factor A - variety, in last place being the interaction between the two factors, variety - fertilization system.

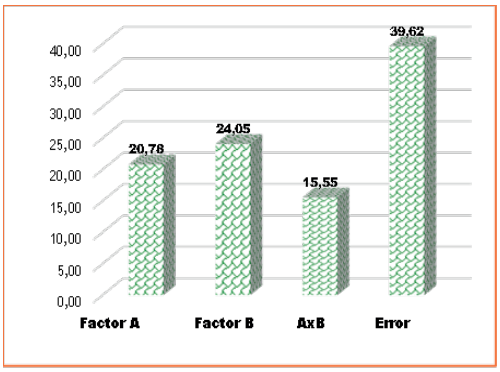


Figure 3. The contribution of factors and the interaction of factors on protein content

CONCLUSIONS

The average of the two years study for the protein content is 14.75%. The highest values of this index were obtained by the varieties Tika Taka - 15.78%, Tiberius - 15.69%, Ciprian - 15.68% and Chevignon - 15.34%. Below the average of the experience, with the lowest values, Rubisko - 14.08%, Sacramento - 13.95% and Centurion - 13.75% varieties were ranked. Regarding the fertilization levels and types of nitrogen, the highest value of 15.36% was obtained at V3 (170 kg ha^{-1} a.s. nitric nitrogen, followed by V1 (120 kg ha^{-1} a.s. nitric nitrogen) - 15.32% and V2 (150 kg ha^{-1} a.s. nitric nitrogen) - 15.27%.
The variants in which fertilization was carried out with ammoniacal nitrogen were ranked below the average of the experience with values of 14.04 - V4 (120 kg ha^{-1} a.s. ammoniacal nitrogen), 14.20 - V5 (150 kg ha^{-1} a.s. ammoniacal nitrogen and 14.32% - V6 (170 kg ha^{-1} a.s. ammoniacal nitrogen). Influence of the A × B interaction on the protein content: no significant differences were recorded.
Wheat mainly uses nitric nitrogen, under the influence of microorganisms, being immediately available and easily absorbed by plants, much more mobile in the soil, which leads to its solubilization in the root zone, especially on sandy soils or in conditions of abundant precipitation. In soil, nitric nitrogen moves mainly by mass flow with water movement and partly by diffusion, while

ammonia nitrogen moves mainly by diffusion and less by mass flow (Mooshammer et al., 2014). The rate of movement of soil solution through mass flow depends on plant transpiration rate, soil water content, and soil texture (Barraclough et al., 2014). Absorption of ammoniacal nitrogen occurs specifically through the root plasma membrane, which serves as an ammonium ion transporter. These uptake transporters have a high affinity for ammonium ions, allowing wheat plants to achieve this even in the presence of low external ammonium concentrations. And once used, ammonium can be directly assimilated into organic compounds through the process of glutamate dehydrogenase (GDH). This process catalyzes the formation of glutamate from ammonium and 2-oxoglutarate. However, the efficient uptake mechanism may not have positive effects on wheat plants that are exposed to excessive soil ammonium levels, causing toxicity such as stunted root growth and reduced nutrient uptake efficiency (Ijato et al., 2021). Several studies have tested the molecular mechanisms behind the physiological responses of wheat plants to ammonia nitrogen fertilization. It has been found that NH_4^+ application can decrease proton efflux lipid peroxidation and protein carbonylation in root tissues, consequently causing plant growth suppression (Du et al., 2021). This is associated with the rate of ammonium absorption exceeding the rate of assimilation in the plant tissues, which leads to the disturbance of ion homeostasis, especially in potassium ions (Xiao et al., 2023). This disturbance of potassium ion balance can appear as leaf chlorosis, leaf scorch and lower biomass production. In addition, ammonium toxicity leads to damage to the chloroplast ultrastructure, which in turn affects photosynthetic activity and plant growth (Castro et al., 2022).

ACKNOWLEDGEMENTS

This work was carried out with the support of University of Life Sciences "King Mihai I" from Timișoara.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

Carthamus tinctorius L. - A SPECIES WITH POTENTIAL FOR EXPANDING CULTIVATED AREAS

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Abstract

Carthamus tinctorius L. – safflower - is a promising oilseed crop for drought-prone regions. Research conducted in the southwestern Banat region on eutric semicarbonatic alluvial soil involved two experiments: one focusing on fertilization and the other on plant density and row spacing. In the fertilization trial, the highest yield of 3335 kg/ha was achieved with an N150P100K60 treatment. This regimen also enhanced grain quality, as evidenced by an increase in the thousand seed weight from 30.4 g in the control to 37.2 g, and an improvement in hectoliter mass from 40.2 kg/hl to 46.2 kg/hl. In a separate experiment evaluating plant density and row spacing, the optimal configuration was found to be 250,000 harvestable plants per hectare with a row spacing of 70 cm, resulting in a yield of 3050 kg/ha. Furthermore, chemical analyses revealed that oil content increased from 30.8% in the control to 40.5% in the $N_{100}P_{100}K_{60}$ treatment, culminating in a maximum oil production of 1398 kg/ha. These results underscore the potential of safflower as a high-yield, high-quality oil crop when appropriate agronomic practices are applied.

Key words*: evolution, milk production, NW Region, Romania, trends.*

INTRODUCTION

In recent decades, the frequency of years with large climatic deviations, which affect agricultural harvests over large geographical areas and large areas of our country, has determined that a good part of the research activity has moved in this direction (Smuleac et al., 2000). In our country, the pedological and atmospheric drought as well as the particularly high temperatures during the vegetation period have caused large harvest losses for economically important crops in our country. The present research falls in the direction of correcting the structure of crops in drought-affected areas, with more resistant crops (Chițu et al., 2024). Among these important crops we can also mention the safflower.

Carthamus tinctorius L., commonly known as safflower, is an ancient oilseed crop belonging to the Asteraceae family. Cultivated for over 4,000 years, safflower has historically been used for its brightly colored petals as a natural dye, its seeds for oil extraction, and its medicinal properties in traditional medicine (Dajue & Mündel, 1996). In modern agriculture, safflower is gaining renewed interest due to its adaptability to arid and semi-arid climates, making it a valuable crop for sustainable farming in water-limited regions (Fernández-Martínez et al., 2015; Imbrea et al., 2017).

Safflower is primarily grown for its high-quality oil, which is rich in unsaturated fatty acids, particularly oleic and linoleic acids (Weiss, 2000). These properties make safflower oil a desirable alternative for culinary, pharmaceutical, and cosmetic applications (Ashrafi & Razmjoo, 2010). Additionally, safflower meal, a byproduct of oil extraction, serves as a protein-rich feed for livestock (Koutroubas et al., 2009). The petals, which contain bioactive compounds such as flavonoids and polyphenols, have been traditionally used in herbal medicine and are now being explored for their potential antioxidant, anti-inflammatory, and cardiovascular benefits (Li et al., 2018). More recently, safflower's industrial applications, including its use in biofuel production and natural dyes, have expanded its economic significance (Singh & Nimbkar, 2016).

Optimal fertilization practices play a crucial role in maximizing safflower yield and oil content. As a deep-rooted crop, safflower efficiently

utilizes soil nutrients but responds positively to nitrogen (N), phosphorus (P), and potassium (K) supplementation (Emongor, 2010). Nitrogen is essential for vegetative growth and biomass accumulation, while phosphorus enhances root development and seed production (Sabaghpour et al., 2018). Potassium contributes to stress tolerance and overall plant health (Hussain et al., 2022). However, excessive nitrogen application can delay flowering and increase susceptibility to lodging, necessitating a balanced fertilization strategy (Ghanbari Malidarreh et al., 2020). Recent studies highlight the potential benefits of integrating organic amendments, biofertilizers, and precision nutrient management to improve safflower productivity in diverse agroecological conditions (Yasin Ashraf et al., 2016).

With growing global demand for sustainable oil crops, safflower presents promising opportunities for expansion and innovation. Future research should focus on breeding high-yielding and climate-resilient safflower varieties, optimizing fertilization techniques to enhance nutrient use efficiency, and exploring the crop's potential in regenerative agriculture (Fernández-Martínez et al., 2015). Additionally, advancements in biotechnology, such as genetic modification and genome editing, may contribute to improved drought tolerance, pest resistance, and enhanced oil composition (Bătrâna et al., 2021, Ahmadzadeh et al., 2021). As agricultural systems shift toward sustainability, integrating safflower into crop rotation and intercropping systems could further enhance soil health, biodiversity, and farm profitability (Zaman et al., 2019).

MATERIALS AND METHODS

The research was conducted in the Southwest of Banat, in the Bozovici Depression near the locality of Pătaș. The experiment was a bifactorial design with 3 replications. Factor A involved fertilization with phosphorus and potassium, with the following levels: a1 – P_0K_{60}, a2 – $P_{50}K_{60}$, and a3 – $P_{100}K_{60}$; while Factor B consisted of nitrogen fertilization, with the levels: b1 – N_0, b2 – N_{50}, b3 – N_{100}, and b4 – N_{150}.

Research on crop density (number of harvestable plants per hectare) and row spacing is an important aspect of cultivation technology - even for species with a high degree of branching, as in the case of safflower, which forms 14 to 60 branches, each ending in a capitulum in which 15 to 20 achenes are produced.

The experiments were carried out in the same area as the previous study during the experimental cycle 2022-2024. This experiment also followed a bifactorial design with 3 replications. Factor A was crop density, with two levels: a1 – 200,000 harvestable plants per hectare and a2 – 250,000 harvestable plants per hectare. Factor B was row spacing, with three levels: b1 – 25 cm, b2 – 50 cm, and b3 – 70 cm. The preceding crop was autumn wheat, and the safflower variety used was CW99OL. Fertilization was applied uniformly using an $N_{150}P_{80}K_{60}$ treatment. The technology implemented in the experimental field was similar to that used in the previous experiment.

For the climatic characterization, data from the Bozovici Meteorological Station were utilized. Analysis of the climatic data indicates that the reference area falls within a moderately temperate continental climate. The limited sub-Mediterranean and oceanic influences impart a distinctive nuance to the depression. The geographical location, topography, and certain local factors give the area its unique climatic dynamics.

The soil in the experimental field is an eutric semicarbonatic alluvial soil. The groundwater table is located at a depth of 3 to 5 meters. The soil texture is that of a coarse clay-loam in the 0-120 cm layer. The soil reaction is slightly alkaline, and the humus content is moderate in the 0-50 cm layer.

The field experiments aimed to optimize fertilization and sowing technology. The safflower variety used was CW99OL, with autumn wheat as the preceding crop. Fertilization with phosphorus and potassium was applied during the autumn plowing, while nitrogen fertilizers were used during the preparation of the seedbed. Soil operations were carried out using conventional methods in the following sequence: disking the soil to destroy the turf and retain moisture; plowing with a moldboard plow to a depth of 23-25 cm; and in spring, preparing the seedbed with a disc cultivator combined with a harrow. Sowing was performed using seed with a purity of 98% and

a germination rate of 77%, at a time when the soil temperature at a 5 cm depth reached 5°C. A seeder designed for cereals was used for sowing, and the soil was subsequently harrowed. Weed control was managed with pre-emergence herbicide applications using Trifluralin and Ethafluralin, followed by post-emergence treatment with Sethoxylin and an additional spray during the vegetative stage.

Safflower can be affected by a number of diseases, including alternariosis, safflower rust, septoria, fusarium, and gray rot. Among the most common pests are the earth beetle, the safflower midge, the seed bug, and the cotton capsule caterpillar. In the research conducted in the reference area, the incidence of these diseases did not warrant treatment as they remained below the damage threshold. However, an outbreak of earth beetle occurred, for which a treatment with approved insecticides was applied. Harvesting was carried out at full maturity in September, when there was no risk of the achenes shattering.

RESULTS AND DISCUSSIONS

The synthesis of yield results obtained during the 2022-2024 experimental cycle depending on fertilization is presented in Table 1.

Table 1. Synthesis of yield results obtained during the 2022-2024 experimental cycle depending on fertilization

NPK Dosage	B factor – nitorogen fertilization				A factor average			
	N_0	N_{50}	N_{100}	N_{150}	yield kg/ha	%	Diff Kg/ha	Sign.
P_0K_{60}	1520	1943	2405	2660	2137	100		
$P_{50}K_{60}$	1716	2278	2755	2941	2422	113	285	X
$P_{100}K_{60}$	1880	2080	3044	3335	2584	121	447	XX
DL 5%= 203 DL 1%= 346 DL 0.1%=672								

	B factor average			
	N_0	N_{50}	N_{100}	N_{150}
Yield (kg/ha)	1705	2100	2734	2985
%	100	123	160	175
Diff. (Kg/ha)		395	1029	1280
Sign.		XX	XXX	XXX
DL 5% = 203 DL 1% = 297 DL 0.1% = 397				

Regarding the impact of phosphorus (P) and potassium (K) fertilization on yield, it is observed that Factor A, representing different doses of phosphorus and potassium fertilization (P and K), significantly influences yield levels. In the control variant (P_0K_0, without P and K fertilization), the lowest average yield was obtained (2,137 kg/ha). The application of phosphorus and potassium ($P_{60}K_0$) led to an increase in yield to 2,422 kg/ha, indicating a positive influence of these essential nutrients. A combined fertilization with phosphorus and potassium ($P_{60}K_{60}$) had the greatest impact on production, reaching 2,584 kg/ha, confirming the crucial role of these nutrients in yield improvement.

The influence of nitrogen doses on yield highlights that the control variant without nitrogen (N_0) results in lower yields (1,520 kg/ha in the P_0K_0 variant). Increasing the nitrogen dose (N_{30}, N_{60}, N_{90}) has a direct effect on yield enhancement, with the highest production recorded at the maximum nitrogen dose (N_{90}), where the yield reaches an average of 2,985 kg/ha. The differences between nitrogen fertilization levels are statistically significant (XXX for LSD 0.1%), confirming the importance of nitrogen as a limiting factor in achieving high yields.

The analysis of the interaction between fertilization factors indicates that the combined use of nitrogen, phosphorus, and potassium leads to the highest yields. The highest production was obtained in the $P_{60}K_{60}$ - N_{90} variant, indicating a synergistic effect among these nutrients.

In conclusion, P and K fertilization significantly contributes to yield increase, with the maximum effect observed in the combined variant $P_{60}K_{60}$. Nitrogen is a critical factor in yield enhancement, with the maximum dose (N_{90}) generating the highest yield increase. The optimal fertilization strategy recommended based on these results is the combination of $P_{60}K_{60}$ doses with the application of a high nitrogen dose (N_{90}) to achieve the best yields. The statistically significant differences confirm the validity of the results, supporting the implementation of optimal fertilization strategies in agricultural practice.

The effect of different nitrogen (N) doses on the thousand-kernel weight (TKW) in relation to phosphorus (P) and potassium (K) fertilization is presented in Figure 1.

The overall trend of thousand-kernel weight (TKW) in relation to fertilization shows a consistent increase in TKW as the nitrogen dose increases across all fertilization variants. The highest TKW values are obtained for the maximum nitrogen doses (N_{150}), indicating a positive influence of nitrogen on grain weight.

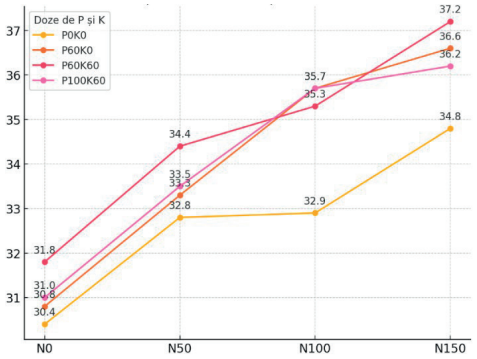


Figure 1. Effect of different nitrogen (N) doses on the thousand-kernel weight (TKW) in relation to phosphorus (P) and potassium (K) fertilization

The impact of phosphorus and potassium on TKW shows that in the absence of P and K fertilization (P_0K_0), TKW has the lowest values, regardless of the nitrogen level applied. A significant increase in TKW is observed in the fertilized variants $P_{60}K_0$ and $P_{60}K_{60}$, suggesting that phosphorus and potassium contribute to improving grain weight. The highest TKW value (37.2 g) was recorded in the $P_{60}K_{60}$ + N150 variant, confirming the beneficial interaction between nitrogen, phosphorus, and potassium.

The effect of nitrogen on TKW highlights that in all variants, nitrogen application leads to a progressive increase in TKW. From N_0 to N_{50}, there is a relatively moderate increase, but after the application of higher doses (N_{100} and N_{150}), TKW increases significantly, demonstrating the importance of nitrogen in the formation of larger and heavier grains. The most pronounced increase in TKW is observed in the variants fertilized with phosphorus and potassium ($P_{60}K_{60}$ and $P_{100}K_{60}$), suggesting that the combined application of these nutrients maximizes nitrogen use efficiency.

In conclusion, nitrogen fertilization has a direct and positive effect on grain weight, with the highest values recorded for the N_{150} dose. The combination of nitrogen with phosphorus and potassium optimizes results, with the $P_{60}K_{60}$ and $P_{100}K_{60}$ variants achieving the highest TKW values. To maximize yield and grain quality, a balanced application of nitrogen, phosphorus, and potassium is recommended, while avoiding over-fertilization, which may reduce the economic efficiency of the crop. These results are essential for establishing an optimal fertilization strategy, ensuring both high yields and superior grain quality.

The effect of fertilization on test weight (TW) [kg/hl], an important indicator of production quality, is presented in Figure 2.

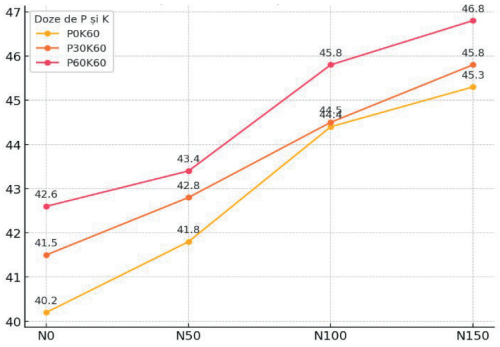


Figure 2. The effect of fertilization on test weight (TW) [kg/hl]

The overall trend of test weight (TW) in relation to fertilization shows a progressive increase in TW with nitrogen application, indicating that fertilization positively influences grain density and quality. The lowest TW values are recorded for N_0, while the highest values are obtained for N_{150}, confirming the importance of applying an adequate nitrogen dose.

In the P_0K_{60} variant, TW values are lower across all nitrogen levels, suggesting that an insufficient phosphorus supply affects seed density. A more balanced fertilization with phosphorus and potassium ($P_{30}K_{60}$ and $P_{60}K_{60}$) leads to a significant increase in TW, with the highest effects observed in the $P_{60}K_{60}$ variant. The highest TW value (46.8 kg/hl) was obtained in the $P_{60}K_{60}$ + N_{150} variant, confirming that the interaction between nitrogen, phosphorus, and potassium optimizes production quality.

In all variants, nitrogen application resulted in an increase in test weight. Compared to the control variant (N_0 to N_{50}), TW increase is moderate, but after the application of higher nitrogen doses (N_{100} and N_{150}), a significant improvement in test weight is observed. The most pronounced increase occurs in the $P_{60}K_{60}$ variant, suggesting that the combined application of nitrogen with phosphorus and potassium enhances the metabolic efficiency of plants, leading to higher grain density.

Regarding this parameter as well, it can be affirmed that nitrogen significantly contributes

to increasing test weight, being essential for the formation of denser and higher-quality grains. Phosphorus and potassium have a complementary effect, optimizing nitrogen efficiency and leading to higher TW values. The best fertilization strategy for maximizing production quality is the balanced application of N_{150} + $P_{60}K_{60}$. Excessive nitrogen fertilization without an adequate phosphorus and potassium supply may not provide the same benefits, emphasizing the importance of balanced plant nutrition.

The evolution of oil content (%) in relation to fertilization, averaged over the experimental cycle, is presented in Figure 3.

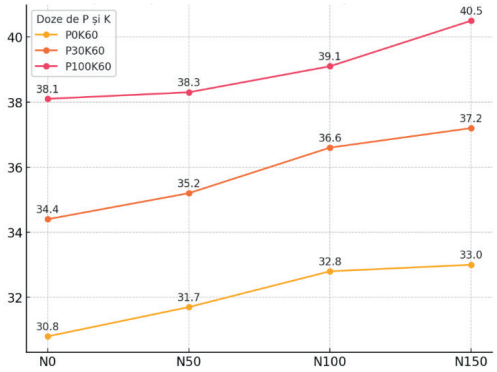


Figure 3. Oil content in relation to fertilization

A gradual increase in oil content is observed with nitrogen application, regardless of the phosphorus and potassium fertilization variant. The lowest oil content values are associated with minimal fertilization (P_0K_{60}, N_0: 30.8%), while the highest values are obtained under full fertilization ($P_{100}K_{60}$, N_{150}: 40.5%).

The impact of phosphorus and potassium on oil content highlights that in the P_0K_{60} variant, oil content increases slowly from 30.8% (N_0) to 33.0% (N_{150}). At the $P_{30}K_{60}$ level, the values are higher, starting at 34.4% (N_0) and reaching 37.2% (N_{150}). In the $P_{100}K_{60}$ variant, which benefits from the best phosphorus and potassium fertilization, the highest oil content values are recorded, ranging from 38.1% (N_0) to 40.5% (N_{150}). These data suggest that phosphorus and potassium application improves nitrogen use efficiency, promoting oil accumulation in seeds. Nitrogen application results in a progressive increase in oil content; however, this effect is more pronounced in variants with higher levels of P and K. The steepest increase is observed in the $P_{100}K_{60}$ variant, indicating that a balanced application of nitrogen together with P and K optimizes oil biosynthesis in seeds. At high nitrogen doses (N_{150}), oil content increases more slowly in variants with reduced fertilization (P_0K_{60}), suggesting that nitrogen alone is insufficient to maximize oil content without an adequate phosphorus and potassium supply.

In conclusion, oil content increases with fertilizer application, but the best combination for maximizing this parameter is the use of nitrogen together with high doses of P and K ($P_{100}K_{60}$, N_{150}). Phosphorus and potassium play an essential role in oil synthesis, and without them, nitrogen efficiency is reduced. To obtain an oil-rich yield, a balanced fertilization strategy is recommended, incorporating nitrogen, phosphorus, and potassium. Furthermore, nitrogen-only fertilization does not maximize oil content, highlighting the necessity of complete plant nutrition for optimal results.

The oil yield, calculated based on grain yield and oil content, is presented in Figure 4.

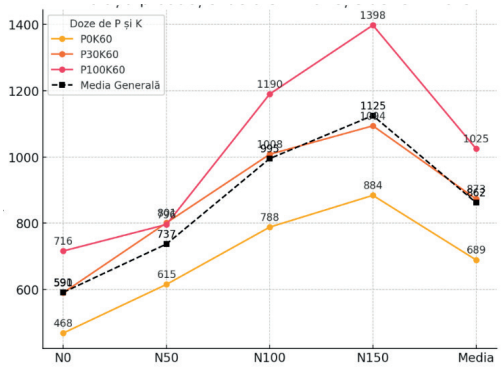


Figure 4. Evolution of oil yield (kg/ha) in relation to fertilization

The data indicate a significant increase in oil yield with the application of higher nitrogen doses, regardless of the phosphorus and potassium fertilization variant. The overall average oil yield increases from 591 kg/ha (N_0) to 1125 kg/ha (N_{150}), confirming the critical role of nitrogen in production efficiency.

At all nitrogen levels, P and K fertilization leads to a significant increase in oil yield. The P_0K_{60} fertilization level (without additional phosphorus) has the lowest oil yield values, with an average of 689 kg/ha. The $P_{30}K_{60}$ level shows

an improvement, reaching an average of 873 kg/ha, demonstrating that phosphorus supplementation enhances nitrogen use efficiency. The $P_{100}K_{60}$ level provides the best results, with an average yield of 1025 kg/ha, and at the maximum nitrogen dose (N_{150}), the highest oil yield is achieved: 1398 kg/ha. This trend confirms that balanced phosphorus and potassium fertilization maximizes nitrogen efficiency in oil biosynthesis.

Regarding the influence of nitrogen, it is observed that at N_0, the lowest oil yield values are recorded, indicating that insufficient nitrogen supply limits biomass accumulation and, consequently, oil production. At the N_{50} dose, a significant increase in oil yield is observed, but the most substantial effects appear at N_{100} and N_{150}. The maximum nitrogen dose (N_{150}) results in the highest oil yields across all P and K fertilization variants, confirming that high-dose nitrogen application is crucial for maximizing oil production.

In conclusion, oil yield significantly increases with fertilizer application. However, the best fertilization strategy for maximizing oil production is the combination of nitrogen with high doses of phosphorus and potassium ($P_{100}K_{60}$, N_{150}). Nitrogen is essential for maximizing oil yield, but without an adequate phosphorus and potassium supply, its efficiency is reduced. The optimal fertilization strategy is $P_{100}K_{60} + N_{150}$, which generated the highest oil yield (1398 kg/ha).

Research on the influence of plant density and row spacing on the yield of *Carthamus tinctorius* L. is presented in Table 2.

Table 2. The Influence of plant density and row distance on the yield of *Carthamus tinctorius* L.

A factor - Density	B Factor – Row distance			A factor average			
	25	50	70	Yield (Kg/ha)	%	Diff (Kg/ha)	Sign.
200k pl/ha	2024	2516	2610	2383	100		
250k pl/ha	2210	2775	3050	2670	112	295	X
DL 5%= 196	DL 1%= 356	DL 0,1%=562					

	B factor average		
	25	50	70
Yield (Kg/ha)	2117	2645	2830
%	100	125	133
Diff. (Kg/ha)		528	713
Sign.		XXX	XXX
DL 5%= 203	DL 1%= 297	DL 0,1%=397	

The results show that at a plant density of 200,000 plants/ha, the yield ranges between 2,024 kg/ha and 2,610 kg/ha, depending on row spacing. At a density of 250,000 plants/ha, the yield is higher, varying between 2,210 kg/ha and 3,050 kg/ha. The mean value for Factor A indicates an increase in yield at the higher plant density (2,670 kg/ha compared to 2,383 kg/ha), with a significant difference of 295 kg/ha. These results suggest that a higher plant density favors increased safflower (*Carthamus tinctorius* L.) production.

The influence of row spacing on yield shows a significant production increase as row spacing widens: at 25 cm: 2,117 kg/ha, at 50 cm: 2,645 kg/ha, and at 70 cm: 2,830 kg/ha. Regarding significant differences, yield increased by 528 kg/ha at 50 cm compared to 25 cm and by 713 kg/ha at 70 cm compared to 25 cm. These results suggest that increasing row spacing contributes positively to yield improvement, possibly due to reduced competition between plants for resources.

The interaction between factors highlights a favorable combined effect of plant density and row spacing. At high density (250,000 plants/ha) and 70 cm row spacing, the highest yield was obtained (3,050 kg/ha). At the lower density (200,000 plants/ha) and 70 cm row spacing, the yield was lower (2,610 kg/ha) but remained superior to the values recorded at narrower row spacings.

CONCLUSIONS

Following the analyses conducted on various aspects of safflower (*Carthamus tinctorius* L.) cultivation, including total yield, thousand-kernel weight (TKW), test weight (TW), oil content, and oil yield, the following conclusions can be drawn regarding the effects of nitrogen (N), phosphorus (P), and potassium (K) fertilization:

- Nitrogen fertilization significantly contributes to improving crop yield. Nitrogen application leads to a progressive increase in total yield, both in terms of grain production (kg/ha) and oil content. The highest TKW and TW values were obtained at the maximum nitrogen dose (N150), confirming the crucial role of nitrogen in optimal plant development and seed quality. In all experimental variants, increasing the nitrogen dose resulted in a substantial rise in oil yield (kg/ha), with the highest values recorded at N_{150}.

- Phosphorus and potassium application optimizes nitrogen use efficiency. P and K fertilization led to significant production increases, demonstrating that while nitrogen is essential, it requires the support of other nutrients to maximize yield. The lowest values for all measured indicators were recorded in the P_0K_0 variant (without P and K fertilization), emphasizing the importance of complete plant nutrition. The best fertilization strategy was the combination of a high nitrogen dose (N_{150}) with $P_{60}K_{60}$ or $P_{100}K_{60}$, where the highest values for total production and seed quality were achieved.
- The interaction between N, P, and K maximizes oil yield. Oil content (%) progressively increased with fertilization, reaching maximum values in the $P_{100}K_{60}$ + N_{150} variant. The highest total oil yield (kg/ha) was obtained in the $P_{100}K_{60}$ + N_{150} variant (1,398 kg/ha), confirming that balanced fertilization is essential for high yield and superior crop quality. The application of nitrogen without P and K led to limited increases in oil content, demonstrating that nitrogen-only fertilization is insufficient for achieving maximum oil production.
- The impact of plant density and row spacing on seed production was also significant. The highest yield was obtained at a density of 250,000 plants/ha with a row spacing of 70 cm.

The obtained results confirm that balanced fertilization with nitrogen, phosphorus, and potassium has a direct and significant impact on the yield and quality of safflower cultivation. The N_{150} + $P_{100}K_{60}$ fertilization strategy is optimal for maximizing both total production and oil content, making it a recommended practice for farmers seeking high-yield and high-quality safflower crops.

These conclusions are relevant for optimizing fertilization technologies and improving the economic efficiency of oilseed crops, with important implications for the development of sustainable agriculture.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

VARIABILITY OF MORPHOLOGICAL TRAITS IN ROMANIAN WILD THYME POPULATIONS

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Abstract

The wild species of the genus Thymus reveal a great taxonomic diversity, indicating a natural evolution that has allowed the adaptation to different habitats and has created the premises of a divergent evolution based on a great genetic variability. The study aims at quantitative morphological characterization by quantifying the associative relationships between the morphological parameters studied, for thirteen thyme populations growing wild in western Romania. Five morphological parameters were used to evaluate the quantitative morphological characters to establish the degree of phenotypic similarity between the studied populations. Depending on the phenotypic similarity, the populations were hierarchically classified into three main clusters using the UPGMA multiparametric cluster analysis. Concerning the analysis of variance for the morphological traits studied in the thyme populations, high and significant values of variance were recorded in the case of leaf length which shows a high capacity for differentiation between the three groups. The lowest diversity between populations of different clusters was observed for leaf width. The overall results revealed high intraspecific and interspecific variability in wild populations of thyme and provided significant information for plant breeding.

Key words*: polymorphism, Thymus pannonicus, Th. dacicus, Th. glabrescens, Th. pulegioides.*

INTRODUCTION

Family *Lamiaceae* (*Labiatae*), is a well-known angiosperm family, comprising of more than 200 genera, many of them being of medicinal (Nieto, 2017; Gurita et al., 2019; Sitarek et al., 2020) aromatic (Karpinski, 2020; Raja, 2021; Butta et al., 2023) and nutritive interest (Carović-Stanko et al., 2016). Within the *Lamiaceae* family, the *Thymus* genus is distinguished, characterized by a great ecological plasticity, including about 250 taxa (species and subspecies), spread all over the world in varied biotopes, the centre of origin being considered the West Mediterranean Region (Jalas, 1971; Hartvig, 1987; Morales, 2002). In the Romanian flora, 18 species of the genus *Thymus* are mentioned, 17 spontaneous and one cultivated species (Sârbu et al., 2013). The spontaneous species in Romania belong with one exception (*T. zygioides* - Section *Hypodromi*, subsection *Serpyllastrum)*, section *Serpyllum*, being mostly classified in the last 4 subsections (Beicu et al., 2023). The taxa of these subsections pose the greatest taxonomic problems, as the species here evolved from the species of the Mediterranean basin centre of origin after the last glaciation, being therefore considered of newer origin and still in a dynamic evolutionary process (Pitarokili et al., 2014; Bălășoiu et al., 2024).

The species of the genus *Thymus* reveal a high variability, both from a morphological (Ložiene, 2006; Dajic-Stevanovic et al., 2008; Baczek et al., 2019), genetical (Thompson, 2002; Sostaric et al., 2012; Soorni et al., 2019; Kim et al., 2020; Alqahtani et al., 2020) and biochemical point of view (Hernández et al., 1987; Imbrea et al., 2016; Ložiene & Venskutonis, 2005; Hadian et al., 2014; Rus et al., 2016; Taghouti et al., 2019), resulting from the influence of abiotic factors. Nomenclature of the genus is in a continuous dynamic, has undergone profound changes, and is constantly subject to critical revisions (El-Gazzar & Watson, 1970; Rosello, 1981; Mártonfi, 1997; Bartolucci et al., 2013).

MATERIALS AND METHODS

Thirteen samples were collected for morphological and molecular analyses, representing wild populations of thyme from different areas in south-western Romania. For the thirteen samples, thirty individuals were collected from each sample. Plants were harvested at full maturity during the flowering period, between May and July 2018. Samples were chosen from locations at a distance from each other to avoid harvesting individuals from the same population and were coded according to the locations from which they were collected (Table 1).

Table 1. *Thymus* populations studied

Population	Abbreviation	Location	Voucher	GPS coordinates (degree, minutes, seconds)		
				Altitude (m)	Latitude	Longitude
Th. pannonicus ssp. *auctus*	Th1	Silagiu	VSNH.BUASTM: 1838	192	45. 60703	21.60335
Th. Glabrescens	Th2	Silagiu	VSNH.BUASTM: 1837	191	45.61382	21.61459
Th. pannonicus ssp. *auctus*	Th3	Pojejena	VSNH.BUASTM: 1821	172	44.78258	21.58887
Th. pulegioides ssp. *chamaedrys*	Th4	Pojejena	VSNH.BUASTM: 1822	194	44.78452	21.59027
Th. pulegioides ssp. *montanus*	Th5	Pojejena	VSNH.BUASTM: 1823	170	44.78465	21.58705
Th. pulegioides ssp. *montanus*	Th6	Pojejena	VSNH.BUASTM: 1824	190	44.78640	21.58897
Th. Dacicus	Th7	Ostrov	VSNH.BUASTM: 1826	70	44.70163	21.62073
Th. Dacicus	Th8	Ostrov	VSNH.BUASTM: 1827	71	44.40145	21.62113
Th. pulegioides ssp. *chamaedrys*	Th9	Caraşova	VSNH.BUASTM: 1832	593	45.15665	21.88147
Th. pulegioides ssp. *pulegioides*	Th10	Caraşova	VSNH.BUASTM: 1831	582	45.15468	21.88160
Th. pulegioides ssp. *montanus*	Th11	Caraşova	VSNH.BUASTM: 1829	511	45.15465	21.87672
Th. pulegioides ssp. *montanus*	Th12	Iabalcea	VSNH.BUASTM: 1833	417	45.22255	21.89523
Th. pulegioides ssp. *pulegioides*	Th13	Nermet	VSNH.BUASTM: 1830	389	45.24185	21.88790

The taxa of the genus *Thymus* were identified based on specialized plant identification books (Sârbu et al., 2013). The voucher specimens (Table 1) were deposited in the herbarium of the Biology Department, University of Life Sciences "King Mihai I" from Timisoara, Romania.

To determine the significance of the differences between the studied genotypes, the processing of the experimental data obtained was done by analyzing the variance and the t-test (two-sample assuming equal variances). Differences were considered significant when p-values < 0.05. Principal component analysis (PCA) was performed using the Euclidean distance, according to Statistica 10.0 (StatSoft Inc., Tulsa, USA).

RESULTS AND DISCUSSIONS

Quantitative morphological characteristics, namely plant height, flower length, leaf length, leaf width, and leaf shape, were recorded and statistically analyzed. Thyme flowers are grouped into inflorescences (racemes); however, for ease of interpretation, the term "flower" will be used.

Plant height in the studied populations exhibited moderate variability (s% = 23.20), with a variation range of 95.2 mm, from 175.90 mm in population Th13 to 80.70 mm in population Th2. High interindividual variability for plant height was observed in populations Th1, Th2, Th3, Th6, Th12, and Th13. Multiple comparisons indicate that populations Th11 and Th13 had significantly greater plant height than the other populations, with differences exceeding 26.1 mm (Table 2).

Regarding flower length, recorded values ranged from 18.05 mm in Th2 to 66.10 mm in Th13, with a variation range of 48.05 mm. Compared to plant height, flower length displayed higher interpopulation variability (40.92%) and pronounced intrapopulation heterogeneity. Populations Th11 and Th13 exhibited significantly longer flowers, exceeding those of the other populations by more than 21.80 mm. The highest interindividual homogeneity for flower length was observed in population Th5, followed by Th4, both from the Pojejena area (Table 2).

Leaf length in the studied populations displayed moderate variability (s% = 16.80), with a range of 6.75 mm, from 8.9 mm in Th6 to 12.65 mm in Th13. As illustrated in Figure 1c, the highest variability and amplitude for this trait were recorded in population Th1 from Silagiu, along with populations from Ostrov (Th7, Th8), whose distributions exhibited a high level of symmetry. Conversely, the lowest intrapopulation

variability was observed in population Th9 from Carașova.

For leaf width, statistically significant differences were observed among the studied populations. Interindividual heterogeneity had a relatively minor impact on the overall results compared to other traits analyzed (Table 3). Values ranged from 1.82 mm in Th1 to 5.85 mm in Th11, with a variation range of 4.03 mm and very high interpopulation variability (41.75%), accompanied by moderate intrapopulation heterogeneity. Pairwise comparisons revealed significantly greater leaf width in populations Th10, Th11, and Th13, with differences exceeding 0.8 mm compared to other populations. Low leaf width values (below 2.5 mm) were observed in populations from Silagiu (Th1, Th2) and Ostrov (Th7, Th8). The highest interindividual homogeneity for leaf width was found in populations Th4, Th6, and Th2, while populations Th10 and Th11 exhibited the greatest intrapopulation variability and amplitude. In contrast, Th9 had moderate variability but a higher amplitude (Table 3).

According to Table 3, leaf shape exhibited the highest variability and amplitude in Th1 and Th2 (Silagiu) and Th8 (Ostrov) populations. In contrast, the lowest intrapopulation variability and amplitude were recorded in Th3, Th4, Th5, and Th6 (Pojejena) populations.

Table 2. Average plants height and flowers length

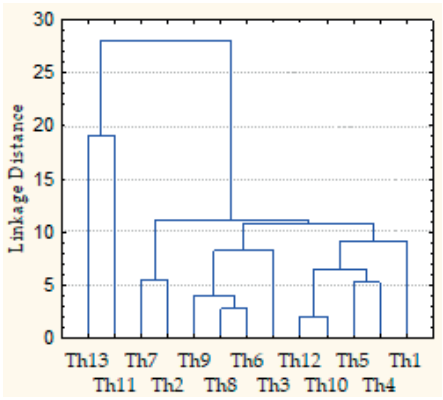
Population	Plant height (mm)		Flower length (mm)	
	$\bar{x} \pm s_{\bar{x}}$	s %	$\bar{x} \pm s_{\bar{x}}$	s %
Th 1	130,80±13,52 b	32,70	42,30±3,40 bc	25,39
Th 2	80,70±6,49 f	25,42	18,05±3,68 f	64,42
Th 3	92,85±6,52 e	22,21	23,85±1,96 e	26,04
Th 4	117,40±5,06 bcd	13,62	31,40±2,50 cde	25,13
Th 5	122,40±5,65 bc	14,59	39,00±1,74 bcd	14,15
Th 6	101,40±6,71 cdef	20,92	26,80±2,58 ef	30,41
Th 7	82,80±4,81 ef	18,37	24,90±3,29 ef	41,76
Th 8	99,50±4,78 def	15,19	29,60±3,19 def	34,09
Th 9	104,00±4,65 cde	14,15	44,30±3,55 b	25,36
Th 10	114,90±6,70 bcd	18,44	33,60±4,81 bcde	45,27
Th 11	156,90±8,29 a	16,71	65,90±9,20 a	44,14
Th 12	115,10±7,64 bcd	20,99	30,10±3,52 de	36,98
Th 13	175,90±13,56 a	32,70	66,10±5,69 a	25,39
	$DL_{5\%}$=21,11 mm		$DL_{5\%}$=11,80 mm	

$\bar{x}$ -mean; $\pm s_{\bar{x}}$ the standard deviation of the mean; s%- coefficient of variability; data sharing different superscripts (a-f) in the same row show statistically significant differences ($p < 0.05$)

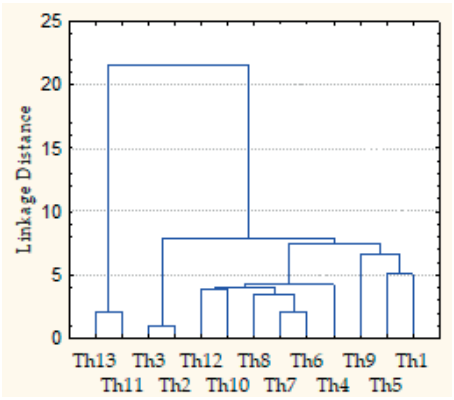
Table 3. Average length, width and shape index of leaves

Population	Leaves length (mm)		Leaves width (mm)		Shape index	
	$\bar{x} \pm s_{\bar{x}}$	s %	$\bar{x} \pm s_{\bar{x}}$	s %	$\bar{x} \pm s_{\bar{x}}$	s %
Th 1	12,15±0,80 ab	20.72	1,82±0,15 ef	26,64	6,08±0,40 ab	20,72
Th 2	10,25±0,57 cde	17.70	1,85±0,08 ef	13,06	5,13±0,29 cde	17,70
Th 3	9,45±0,43 de	14.43	4,12±0,23 bc	17,72	4,73±0,22 de	14,43
Th 4	9,60±0,46 de	15.29	3,35±0,11 d	10,07	4,80±0,23 de	15,29
Th 5	10,25±0,37 cde	11.56	4,00±0,24 cd	18,63	5,13±0,19 cde	11,56
Th 6	8,90±0,43 e	15.40	3,80±0,15 cd	12,71	4,45±0,22 e	15,40
Th 7	12,10±0,95 ab	24.78	2,30±0,17 e	23,37	6,05±0,47 ab	24,78
Th 8	11,80±0,79 abc	21.06	2,15±0,18 e	26,97	5,90±0,39 abc	21,06
Th 9	11,90±0,35 ab	9.25	4,70±0,24 b	16,02	5,95±0,17 ab	9,25
Th 10	9,80±0,61 de	19.72	5,50±0,37 a	20,99	4,90±0,31 de	19,72
Th 11	11,00±0,58 bcd	16.60	5,85±0,49 a	26,74	5,50±0,29 bcd	16,60
Th 12	11,00±0,54 bcd	15.45	4,25±0,29 bc	21,66	5,50±0,27 bcd	15,45
Th 13	12,65±0,47 a	20.72	5,70±0,20 a	26,64	6,33±0,24 a	20,72
	$DL_{5\%}$=1,61 mm		$DL_{5\%}$=0,67 mm		$DL_{5\%}$=0,81	

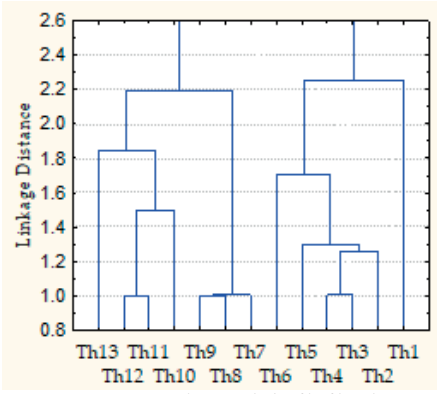
$\bar{x}$-mean; $\pm s_{\bar{x}}$ the standard deviation of the mean; s%- coefficient of variability; data sharing different superscripts (a-f) in the same row show statistically significant differences ($p < 0.05$)



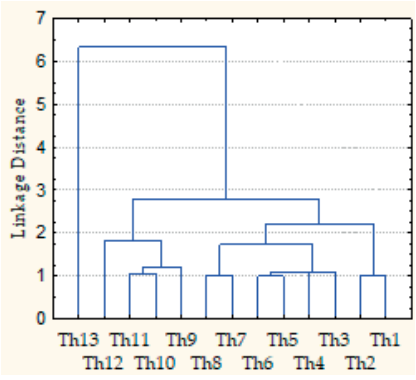
a. Cluster analysis of plant height



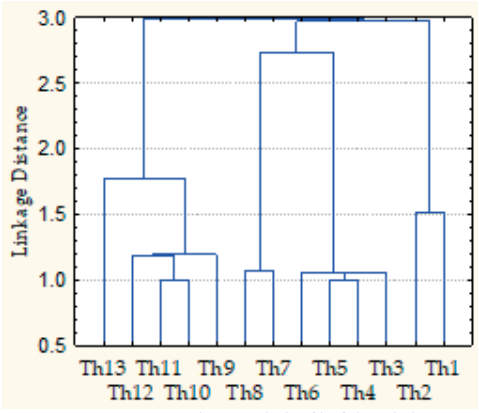
b. Cluster analysis of flower length



c. Cluster analysis of leaf length



d. Cluster analysis of leaf width



e. Cluster analysis of leaf shape index

Figure 1. Cluster analysis based on morphological characters

Based on both morphological similarity for plant height and flower length, all populations were classified into two main clusters. The first cluster groups the two populations with the highest values of these characters, populations Th11 and Th13. The second cluster is more complex, grouping several sub-clusters in which the other populations analysed are included (Figure 1a, 1b).

Hierarchical classification of populations based on similarity of leaf length and width groups them into two main clusters, each with several sub-clusters. A medium variability is observed, with the exception of the Th13 population, which for both characters show the highest values and is differentially represented (Figure 1c, 1d). Regarding the cluster analysis of populations based on similarity of leaf shape, three main clusters can be noticed, as can be seen in the dendrogram shown in Figure 1e.

In terms of the five morphological characters studied (plant height, inflorescence length, leaf length, width and shape), the highest phenotypic similarity exists between Th7 and Th8 populations belonging to the species *Th. dacicus* from Ostrov, at 98.91%. An increased phenotypic similarity can also be observed between the following populations: Th3 - Th6 (98.76%); Th3 - Th4 (97.39%); Th4 - Th5 (97.71%); Th5 - Th12 (97.54%). A pronounced morphological similarity of 91.72% was also observed between populations Th9, Th10, 11 of *Th. pulegioides* collected from Carașova. A high degree of phenotypic differentiation was observed among the populations: Th2 - Th13 (74.59%); Th6 -Th13 (70.07%); Th3 -Th13 (62.81%); Th4 - Th13 (51.26%). As such, it is found that overall, the Th13 population of *Th. pulegioides* from Nermet was morphologically differentiated to a considerable extent of 42.34% compared to the other populations included in the study. A significant phenotypic diversity of 53.92% was also recorded between Th2 and Th11 populations, *Th. panonnicus* (Th1) and *Th. glabrescens* (Th2) populations from Silagiu showed a morphological similarity of 79.12%.

Based on the morphological similarity of the five characters, the populations were hierarchically classified into three main clusters between which there is an average diversity of about 37%. The first cluster consists of the *Th. dacicus* populations from Ostrov, Th 7 and Th8, which are phenotypically similar to approximately 95% of the Th1 population of *Th. pannonicus* from Silagiu and to approximately 89% of the Th9 (*Th. pulegioides*) population from Carașova. The second group consists of Th3, Th4, Th5, Th6 populations belonging to *Th. pulegioides* collected from Pojejena which show a morphological similarity of about 94% to Th10 and Th12 populations of *Th. pulegioides* collected from Carașova. Also included in this group is the Th2 population of *Th. glabrescens* from Silagiu, which show a similarity of about 8% to the other six populations. The Th11 and Th13 populations of *Th. pulegioides*, between which there is a morphological similarity of 91.41%, make up the third cluster, differing by about 20% from the populations in the other clusters (Figure 2).

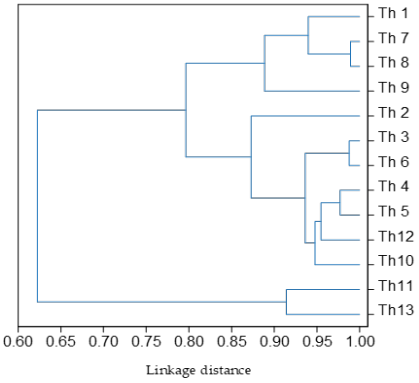


Figure 2. Dendrogram by UPGMA method for morphological traits

Concerning the analysis of variance for the morphological characters studied in the thyme wild populations, it can be observed that high and significant values of variance were recorded in the case of leaf length which shows a high capacity of differentiation between the three clusters. The lowest diversity between populations of different clusters was observed for leaf width (Table 4).

Table 4. Variance analysis for all morphological traits analysed

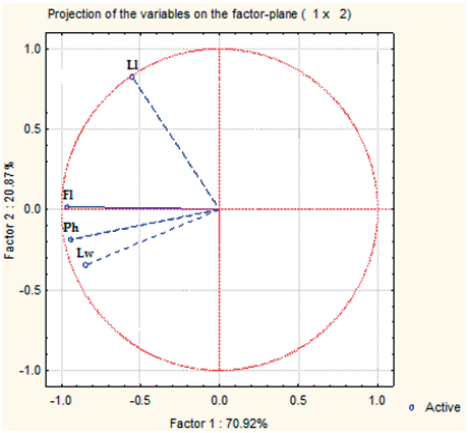
No.	Character	Between		Within		F test
		SP	GL	SP	GL	
1.	Plant height	1,491	1	10,512	11	1,56
2.	Flower length	3,040	1	8,962	11	3,73
3.	Length leaves	9,654	1	2,426	11	43,77**
4.	Width leaves	0,005	1	11,934	11	0,01

GL - degrees of freedom, SP- sum of squares, Significance for F-test: ns p>0.05; *p≤0.05; ** p≤0.01; *** p≤0.001

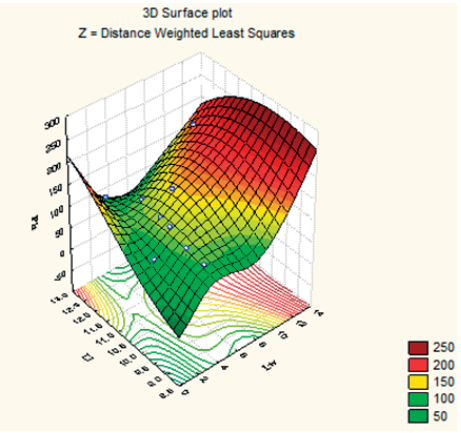
Table 5. Analysis of variance for each population regarding morphological traits

Population	Between		Within		F test
	SP	GL	SP	GL	
Th 1	59.67	1	6.17	3	28.99*
Th 2	52.74	1	1.93	3	82.07**
Th 3	32.06	1	1.70	3	56.47**
Th 4	30.00	1	2.89	3	31.19*
Th 5	31.94	1	2.11	3	45.33**
Th 6	25.58	1	1.83	3	41.90**
Th 7	74.25	1	1.29	3	172.20**
Th 8	64.76	1	2.51	3	77.34**
Th 9	50.02	1	0.36	3	416.18**
Th 10	25.93	1	2.14	3	36.34**
Th 11	22.45	1	1.54	3	43.68**
Th 12	43.21	1	2.41	3	53.73**
Th 13	36.13	1	3.42	3	31.69*

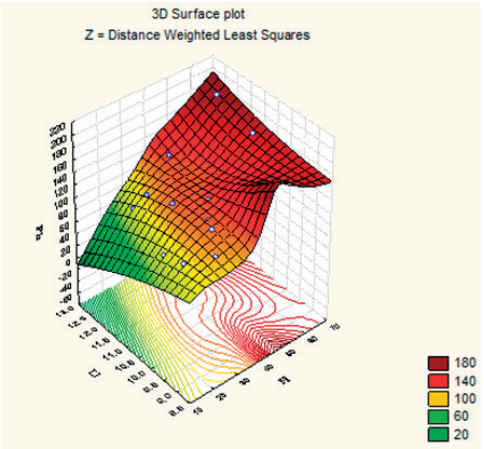
GL - degrees of freedom, SP- sum of squares, Significance for F-test: ns p>0.05; *p≤0.05; ** p≤0.01; *** p≤0.001



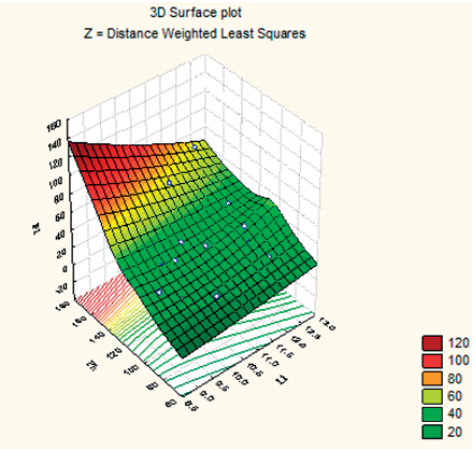
a. CP1 and CP2



b. Length leaves vs width leaves vs. plant height



c. Flowers length vs. length leaves vs. plant height



d. Plant height vs. length leaves vs. flowers length

Figure 3. Projection of the variables for the two main components CP1 and CP2

Regarding the results presented in Table 5, it is observed that the Th7 and Th8 populations belonging to the *Th. dacicus* from Ostrov, generate the highest morphological differences, with high and distinctly significant contributions to the total variability recorded on the basis of

the dendrogram. The lowest contribution to the total variability among the three clusters was observed for Th10 and Th11 populations belonging to *Th. pulegioides* from Caraşova.

At the level of the first cluster the Th1 population has the highest contribution to interpopulation diversity, while at the level of the second cluster the Th4 population stands out. Based on a linear correlation matrix, PCA (Principal Component Analysis) was applied to the mean values of the measured traits to study which parameters contributed the most to total data variation. The PCA produced 4 components. The first two principal components accounted for 70.92% and 20.87% of the variance respectively, for a total of 91.79% (Figure 3 a-d). Component 1 (CP1) includes the variables with the highest correlation coefficients: plant height, flower length, leaf width and leaf length, and component 2 (CP2) includes the correlation coefficient with the highest value, represented by leaf length, as shown in Table 6. The most important variable integrated into the first component was flower length, which was negatively correlated with this component.

The two-dimensional plot based on the two main components CP1 (70.92%) and CP2 (20.87%), expresses 91.79% of the variability of morphological characters. Based on the position of the populations to the vectors of the different characters, the dimensions of the characters were described. It can be seen that Th13 population shows the highest values of all characters (Table 6). Thyme populations show significant morphological variability, with some clustering closely while others are dispersed, indicating indicating phenotypic differences relevant for selection.

Table 6. Matrix of main components

Variable	Factor 1	Factor 2	Factor 3	Factor 4
Plant height	**-0.943068**	-0.189173	-0.219034	0.163889
Flower height	**-0.962191**	0.016169	-0.211304	-0.171106
Length leaves	**-0.550645**	**0.824935**	0.122341	0.036114
Width leaves	**-0.847552**	-0.343815	0.404119	-0.011572

CONCLUSIONS

Distinctive morphological features of thyme populations belonging to the four species found in the wild flora of Banat were identified, showing significant variability for the morphological parameters studied. These differences indicate a major influence of environmental factors in the phenotypic expression of the populations of this species. Statistical analyses allowed the identification of distinct groups with improvement potential. The analysis of plant height and inflorescence are the most influential traits, as taller plants also have longer inflorescences. Populations Th13, Th11, and Th10 can be considered for breeding programs when biomass production is the main objective. The analysis of leaf length indicates that leaf variation is not closely correlated with plant and flower height. This suggests that leaf size can evolve independently, a trait that may be valuable for cultivation. Populations Th9 and Th13, which have large leaves, are ideal for selecting high-yield varieties for biomaterial and essential oil production. The analysis of leaf width is important but does not determine the main variation. Although leaf width contributes significantly, it does not dominate variation in the same way as plant and inflorescence height. Populations Th5 and Th6, which have wider leaves and high genetic stability, can be considered for specific selection. The research results provide a solid scientific basis for breeding programs, optimizing the selection of the most valuable genetic lines. Correlating these results with genetic and chemical analyses is necessary to determine the most valuable spontaneous populations.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

NEW ASPECTS WITH WEED COMPETITION AND CONTROL FROM THE SUNFLOWER CROP

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Abstract

Having a more developed leaf system, one might think that the sunflower can fight much better with weed species during the growing season. Young plants, however, are the most vulnerable, as in the case of other cultivated plants. Weed biomass currently exceeds 10-12 t/ha, and their spectrum is diverse. On average, production losses due to weeding exceed 1.0-1.5 t/ha of seeds. As a structure, MA constituted on average 8.6 t/ha, DA 2.6 t/ha, and DP 1.0 t/ha. Weeding as often as needed has proven somewhat more effective than in the case of other cultivated plants. Herbicide with specific products contributed year after year to obtaining obvious increases in production. In the case of the application of single herbicides, the increases were below 1.0 t/ha, and in the case of the combined and associated ones, the increase in production was around 1.5 t/ha. Today, reducing herbicide doses still requires adequate research.

Key words: *competition, herbicides, hoeing, sunflower, weeds control.*

INTRODUCTION

Sunflower plants get massively weeded (Ionescu & Șarpe, 2001), just like any spring plant sown in sparse rows. Although young sunflower plants experience relatively more obvious growth (Swanton et al., 2015), the degree of weeding that occurs is just as dangerous (Kempenaar et al., 2002). As it is known, the taproot of the plants obviously penetrates the soil and should compete a little better with the segetal species that appear. From practice, it has been proven that this aspect is relative (Silva et al., 2012), and weeding occurs at high levels. And in sunflowers, methods are used to avoid the establishment of unwanted competition (Tagour, 2015). on the one hand, the emergence of weeds is avoided during the emergence of cultivated plants. At this moment, it is good that the seedlings do not get weeded, in this case also avoiding the dangerous stress for the first moments (Ionescu, 2024). on the other hand, 1-2 treatments are used from the sunflower plant vegetation, for the segetal species that continue to appear, through the spring and summer forms (Simiç et al., 2011). as well as those that emerge towards the warm season (generically called summer species), are equally dangerous in the sunflower culture (Chauhan et al., 2012). On the one hand, we opt for the use of effective and specific herbicides, and on the other hand, we resort to 2-3 treatments. Normally the first treatment considered mandatory will resolve both the monocots and dicots spectrum. This treatment is applied in most pre-emergent cases. On the other hand, they are used through 1-2 herbicide treatments in plant vegetation. The most suitable moment for sunflower plants is that of 4-6 leaves, with the presence of weeds. At the same time, weed nets are used for the most effective weed control (Ionescu et al., 2014). These are interspersed among chemical treatments, and their effectiveness has proven to be very good. On the reduced areas of sunflower culture, you can also resort to manual weeding, which has the role of increasing the effectiveness of other weed control measures. Chemical measures, along with mechanical ones, but also others (for example rotations suitable to the farming system practiced) are included among the new rules, accepted by Chauhan et al., 2012). Overall, they provide a more beneficial direction for the integrated management of weeds in sunflower culture. The research carried out to find newer, but also diverse, ways is currently aimed at

prospecting the need to reduce the degree of weeding in this valuable crop plant: the sunflower, including through chemical strategies (Pannacci et al., 2007). The purpose of their promotion is, on the one hand, the avoidance of human effort, the increase of productivity, along with the reduction of the cost price per product unit. Both plant and seed biomass are targeted. The present results, although considered relatively new, further experimentation and specific studies are needed. Thus, investigations of an ecological nature have begun regarding the interrelationships between weed species, the economic thresholds of damage, as well as non-chemical control methods. The present paper presents results obtained from several experimental directions: demonstration of the competition between weeds and sunflower plants, investigation of new, ecological ways by carrying out weeding, along with new chemical methods of weed control (Kudsk, 2003). The results obtained so far support very well the conclusions obtained from the sunflower culture in the area of heavy clay soils in the south of the country.

MATERIALS AND METHODS

The studies whose results are presented in the paper were carried out over a period of several years. Their presentation will be done through specific research series. On the one hand, sunflower weeding was studied, and on the other hand, control methods, both by cultivation and by herbicides. Thus, within the competition between weeds and sunflower plants, weed species formed from the respective botanical categories, known, were determined every year (Chauhan et al., 2012). The natural distribution of weed species included both compact areas, separate areas and even sporadic areas. With the data obtained, both the correlation between the total biomass and the production losses of sunflower seeds were expressed. On the other hand, it was demonstrated how the degrees of natural weeding influenced the growth and development of crop plants. In another multi-year study, the effectiveness of the nets, both mechanical and manual, and the complexity between them was followed. Between the two methods: chemical and non-chemical, comparisons were made for their acceptance at the level of production farms. By combining weeding with weeding, a new study has recently been started to determine the interrelationships, the economic advantages, then the application period, as well as the possibilities of reducing herbicide doses.

In another specific direction studied, reference is made to the exclusive use of herbicides, as chemical means of weed control. On the one hand, the aim was to reduce the degree of weeding, and on the other hand, both the protection of the soil and the protection of the sunflower plants were taken into account. Over the years, permanent progress has been made both by professional companies and by researchers in the field, so that all farmers have at their disposal the most modern, effective and even cheap options. The recommendations also included the possibilities that agricultural producers have for adapting to the new situations that appear every year. In a series of separate experiments, several classic herbicides, as well as newer ones, were studied, with the aim of highlighting the new directions of the European Union. The possibilities of reducing herbicide doses for three products are targeted: alachlor and oxyfluorfen, along with promethryn.

The experimental variants were located in the specific research field of the resort. The placement method was that of the Latin rectangle, in 4 repetitions. The surface of the variants was in all cases 25 m^2. Plant samples, both for weeds and sunflowers, were collected decadal throughout the vegetation period. For the deposition of the dry matter from the sunflower seeds, samples were collected every 5 days. In both cases the samples were collected with the metric frame from all repetitions. The drying process of the samples was carried out classically, at 105°C, for 8 hours (Clawson method). The statistical processing was done according to the variance analysis method (anova test), and the Excel program was used to express the average data. The hybrids used were those grown by farmers in the area, and the applied technology was the one recommended by the resort.

RESULTS AND DISCUSSIONS

Due to the appearance of a whole vegetable carpet from weeds since the first sunflower moments, it was studied from the beginning. On the one hand, the multitude of existing species was taken into account, along with some specific to the natural conditions of the white clay-soil (Ionescu & Popescu, 2024). The wide floristic composition existing in each sunflower growing area, most produce economic, physiological damage to the sunflower. The interaction between weeds and sunflower plants can be researched either according to a more aggressive weed species, or simultaneously for all species in the crop. In the application of means of control of the entire spectrum of weeds, it is recommended that the weeding be considered in all its composition.

The natural composition of sunflower weeds. The weed species observed, noted and weighed from the untreated & unseeded control was diverse, being specific to the sunflower cultivation area (Table 1; Figures 1 and 2). Among the species, annual dicotyledons (DA) dominated, then perennial dicotyledons (DP) and less annual monocots (MA) and perennials (MP) in number. It is also noted that some species from the three categories were problem species, being highlighted in the table.

Table 1. The main weed species from sunflower crop

No.	Perennial dicots	Annual dicots	Annual monocots	Perennial monocots
1.	***Cirsium arvense***	***Amaranthus retroflexus***	***Echinochloa crus-galli***	*Agropyron repens*
2.	***Convolvulus arvensis***	***Chenopodium album***	***Digitaria sanguinalis***	
3.	*Lathyrus tuberosus*	***Raphanus raphanistrum***	***Setaria glauca***	
4.	*Rumex crispus*	***Sinapis arvensis***	***Setaria viridis***	
5.	*Sonchus arvensis*	***Centaurea cyanus***	*Lolium* ssp.	
6.	*Taraxacum officinale*	***Xanthium italicum***	*Poa annua*	
7.		***Ambrosia artemisiifolia***		
8.		***Polygonum hydropiper***		
9.		*Sonchus asper*		
10.		*Hibiscus trionum*		
11.		*Polygonum persicaria*		
12.		*Scleranthus annuus*		
13.		*Matricaria inodora*		

Xanthium italicum, weed-problem



Figure 1. *Ambrosia artemisiifolia* competition



Figure 2. *Centaurea cyanus* competition

After completion of the vegetation period, metric frame samples were collected for all weed species. After weighing them, they were separated into large botanical groups: DP- perennial dicots, DA- annual dicots, MA- annual monocots and MP- perennial monocots. Their quantitative evolution from the untreated and unseeded control was specific (Table 2).

The behavior of the sunflower plants in the naturally weeded control was specific, in the last 12 years (Figure 3). Three groups of data can be seen from the graph. In the first, the sunflower plants produced seeds between 1.2 and 1.5 t/ha, due to somewhat less competition. In a single year, the cultivated plants produced 1.8 t/ha of seeds under these conditions. The third group placed seed productions between 0.7 and 1.2 t/ha. From the graph it can be seen that in most years the sunflower plants were strongly competed for, on the one hand, and on the other

hand there were also years with somewhat less close competition in which the crop plants were less competed for.

Table 2. Weed evolution (t/ha biomass) by botanical gruoups from untreated plot

Years/form	Total t/ha	PD	AD	AM
1	9.9	0.5	4.2	5.2
2	13.7	0.8	3.3	9.6
3	13.0	1.7	0.2	11.1
Mean	12.2	1.0	2.6	8.6
Participation, %	100	8	21	71

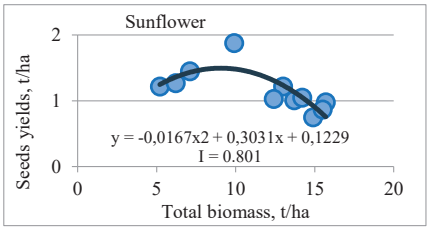


Figure 3. Corelation between weed biomass x sunflower seeds yields from untreated plot (Ionescu, 2014)

Competition between weeds and sunflower plants. The weeding that occurred in the unmaintained sunflower crop (no herbicides, uncultivated) caused significant damage to it (Figure 4).

And in sunflower, the average rate of accumulation (Kandel et al., 2019), of both total and grain biomass, with and without the presence of weeds, showed delays by natural weeding (Figure 4 - 1.A). The deposition of useful substances in the grains was at a level considered low. Thus, through weeding the accumulation of total biomass reached a maximum level at 760 g/m^2, compared to the clean weed culture with a maximum level of 1500 g/m^2. Dry matter accumulation rates of both weeds and sunflower followed specific sinusoidal curves (Figure 4 - 1.B). Sunflower plants without weed competition accumulated a maximum of 48 g/m^2/day. In the presence of weeds, this rate had a slight deviation towards maturity, and the maximum value was 20 g/m^2/day. The respective deviations were followed by the submission of active ingredients in sunflower plants.

The respective curves were influenced by the presence of weeds. The unwanted weed biomass obtained separately experienced a similar rate, but at much higher values, namely by a maximum of 35 g/m^2/day.

Regarding the rate of accumulation (deposition) of active ingredients in sunflower seeds, a differentiation by weeding was found. From the graph (Figure 5), it can be seen that weeding was also harmful for sunflowers, with great repercussions on the formation of production. In the conditions of competition, the rate of accumulation was low and diverted towards the beginning of the deposit process of active ingredients in seeds. As a value, this rhythm had a maximum of 4 g/m^2/day, after which it decreased. Sunflower free by weeds had a maximum accumulation of 9.8 g/m^2/day.

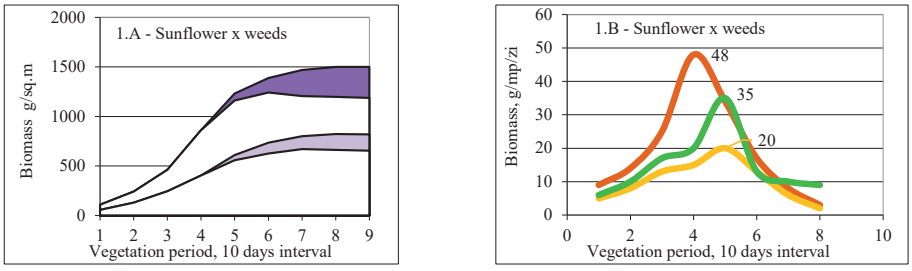


Figure 4. The evolution of biomass- total biomass and grain yield formation (1.A) and weeds biomass, sunflower total biomass and seeds filling rates (1.B), as affected by weeds encroachment or not (brown- sunflower without weeds, yellow- sunflower with weeds, green- weeds. The axis represent consecutive observations with time- intervals of 10 days (after Ionescu, 2005 - 1.A, and Ionescu, 2001 - 1.B)

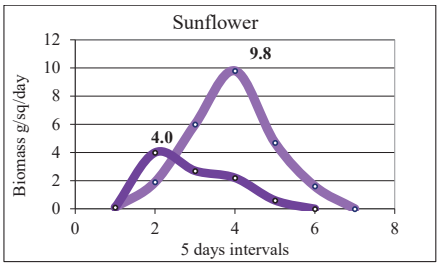


Figure 5. The seeds rate of accumulation, in weed-free and weedy sunflower (after Ionescu, 2012)

Table 3. Efficacy of weeding control (hoed type)

Hoeing type	Seeds yields levels	
	kg/ha	%
Mechanical + manual	3043	100.0
Mechanical	2922	96.0
Manual	2069	68.0
Not hoed	1366	44.9
LSD (5 %)	548	18.0
LSD (1 %)	726	23.9
LSD (0.1%)	936	30.8

Herbicide of the sunflower crop. Among the new rules for weed control in the sunflower culture, three very important directions have been realized: i) competition with sunflower plants, ii) cultivation and iii) the rational use of herbicides. The first two rules have already been presented in the paper. The third direction refers to the use of herbicides.

Today, in the case of the sunflower, single active substances and in more complex formulations are available, sufficient to cover the entire range of weeding situations in this cultivated plant as well. Absolutely all herbicides recommended for sunflowers have both a high degree of effectiveness and a selective protection for crop plants.

At the moment, both companies in the field and research have and can recommend the best weed control options in sunflower culture. Recently, due to the need to emphasize the protection of the agricultural environment, it is recommended to reduce as much as possible the use of herbicides, or exclusively with herbicides (Bostrom., & Fogelfors, 2001). Moreover, the products that have a dangerous residual effect on the agricultural environment were taken out of use and the friendliest ones were promoted.

Today's practice aims to make the best possible use of decisions regarding weeding, with reference to both climatic factors and the spectrum of weeds existing on the respective areas. Considering these two aspects, in practice sometimes herbicides with unilateral application are used. The high level of effectiveness is obtained with the help of herbicides in combination (tank mix), or those associated (Table 4).

Table 4. The efficacy of herbicides in sunflower crop

No.	Herbicides	Doses/ ha	WCD, %	Selectivity (notes)	Production increase, kg/ha
	Single applied herbicides				
1.	Acetochlor 820-840 g	1.75	16	1.0	1070
2.	Alachlor 480 g	6.0	37	1.0	510
3.	Bifenox 480 g	1.5	58	1.3	213
4.	Oxyfluorfen 240 g	1.0	15	1.0	1150
5.	Linuron 475 g	2.5	67	1.0	260
6.	Pendimethalin 330 g	5.0	36	1.0	1060
7.	Promethryn 500 g	5.0	26	1.0	1235
		Mean	**36.4**		**785**
	Combined (tank mixed) and associated herbicides				
1.	Alachlor 480 g + promethryn 500 g	6+4	14	1.0	1307
2.	Alachlor 480 g + oxyfluorfen 240 g	6+1	10	1.0	1329
3.	Alachlor 480 g + fluorochloridon 250 g	6+1.5	25	1.0	990
4.	Butylat 800 g + oxyflueorfen 240 g	6+1	15	1.0	1465
5.	Metolachlor 960 g + linuron 475 g	1.5+2.5	15	1.0	1200
6.	Metolachlor 960 g + fluorochloridon 250 g	1.5+1.5	11	1.0	1330
7.	Metolachlor 960 g+fluorochloridon 250 g + imazamox 40 g	1.5+1.5+1.2	5	1.3	1428
8.	Pendimethalin 330 g + bifenox 480 g	5+1.5	31	1.3	1100
		Mean	**15.8**		**1270**

*WCD- weeds covered degrees

Experiments with the reduction of herbicide doses in crop plants is relatively new (Ionescu, 2011). Recently, the European recommend-dations also invite to find ways to reduce the normal, recommended doses (Zhang et al., 2000). In the present example from the paper, the effectiveness of three treatments is presented, in different doses: 20%, 40%, 60%, 80% and 100%, of the normal, recommended doses (Figure 6).

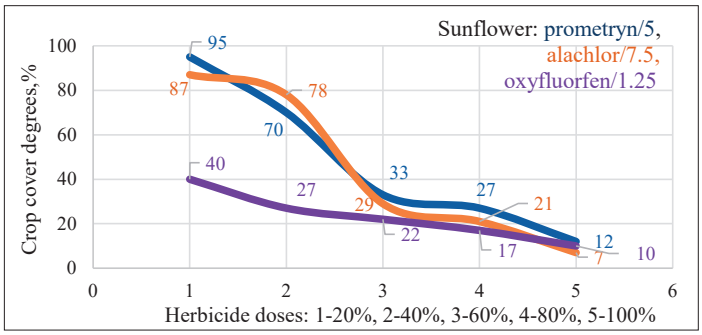


Figure 6. The influence of different doses of herbicides in reducing the weed levels from sunflower crop (Ionescu, 2011)

The evolution of the effectiveness expressed by the degree of weed coverage (GA) left uncontrolled in these different doses demonstrates characteristic situations. Two products: promethryn and alachlor reduced weed cover to acceptable levels only at 60-100% doses. At 60% promethryn reduced WCD to 30-31%, virtually on target of 30%, below which weeds no longer produce significant losses in sunflower production. Alachlor reduced the WCD by an additional 2-3% at 60% dose, demonstrating an additional benefit. The third herbicide, oxyfluorfen reduced WCG the most, even from doses of 20% of normal. In some favorable years, this product can be recommended to be applied in doses of 40-60% of the normal, due to the expression of much increased effectiveness. Research of this kind should be promoted in as many centers as possible with different ecological conditions (Kudsk, 2002). Their purpose is to be able to recommend, at a certain time and crop condition, herbicide products in the lowest possible doses.

On the selectivity of some herbicides applied in sunflower crop. In most sunflower herbicide situations, phytotoxic effects are not produced, as they are selective in their action. However, in a sunflower crop there may be times when a herbicide, usually with post-emergence application, with or without repeating that treatment, can show some symptoms on the plant foliage. In other situations of treatment in too early seedling vegetation, the phenomenon of growth arrest may occur. These phenomena may be favored by less favorable climatic conditions for cultivation, but usually the symptoms disappear after a short period of time (Inoue et al, 2019). Examples of sunflower-induced phytotoxicity can be found in Table 4, for bifenox products with a selectivity grade of 1.3 and imazamox also with a grade of 1.3. In both cases the plants recovered in a short time and developed normally.

CONCLUSIONS

And the sunflower is one of the field plants that get massively weeded in some years. Depending on the area, here too the causes can be: the specific reserve of seeds in the forest soil, along with the equally reduced competition of the seedlings from the moment of emergence.

From the practical point of view of weed control in the sunflower culture, the information about: the competition between sunflower plants and weeds, the effectiveness of cultivation works in reducing this competition, this being considered by Europe to be mild compared to the culture environment, becomes important and using the recommended herbicides as correctly as possible.

From a study it was shown that on average the average level of weeding was 12.2 t/ha of total

biomass, of which annual dicot species (DA) represented 21%, DP 8%, and the largest proportion, 71% were MA.

The production of naturally weeded sunflowers was between the limits of 720 and 1810 kg/ha. The other data fell within these limits. This study demonstrates the fact that in some years the crop can show a certain degree of competitiveness with the weeds present in the crop.

Specific competition between sunflower plants and weeds demonstrated distortions in plant biological performance. Certainly, in the first period of vegetation, the sunflower plants showed a rather strong fight with the weeds, after which it gave way, but not completely.

Manual and mechanical weeding had very high efficacies in sunflower culture. The differences through mechanical weeding were 68.0% of the ideal, and manual weeding 96.0% of the completely clean culture.

Chemical control of sunflower weed species achieves that lack of competition for a sufficient period of time for the plants to grow and develop as normally as possible. Among the control strategies used, single herbicides give good results, but the best solutions were found with associated treatments. Tank-mix treatments can often be used separately, but only in relation to the concrete situations of existing weeding levels.

Reducing herbicide rates requires several years of research, and the resulting recommendations will be advised with caution.

The phenomenon of phytotoxicity can appear on young plants, following treatments carried out with some herbicide products. Climatic conditions can contribute to intensifying the phenomena, but usually they last only a few days, after which the sunflower plants continue their normal growth and development.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

MONITORING OF *Rhagoletis cerasi* WITH THE HELP OF DECIS TRAP TRAPS AND MONILIOSIS IN THE CHERRY ORCHARD FROM USAMV OF BUCHAREST

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Abstract

In the conditions of our country, the cherry fly (Rhagoletis cerasi L.) is the most important pest of cherry orchards. At the same time, species of the pathogen Monilinia laxa, which cause moniliosis and brown rot, produce severe damage with high economic relevance. In the present work, we aimed to present the moment of appearance of adults and their evolution in the crown of cherry trees, based on Decis Trap traps, and how the climatic conditions influenced the activity of the cherry fly. In order to establish the dynamics of cherry fly populations, Decis Trap traps were used, installed in the crown of the trees. It is known that the attack produced by this species significantly affects fruit, production, depreciating the quality of the fruit and can be a way of installing the Monilinia laxa pathogen in the fruit. The incidence of fruit attack was between 6.78% and 32.25%.

Key words*: Rhagoletis cersi, traps, dynamics, Monilinia laxa.*

INTRODUCTION

The study proposed in this work consists of the evolution of the cherry fly (*Rhagoletis cerasi* L.) and how the climatic conditions influenced the activity of this species. It is known that the cherry fly is the most important pest in the cherry orchard, significantly affecting fruit production and quality. The larvae of the cherry fly are the most important pest of the cherry tree, especially in the semi-late and late varieties (Florian et al., 2018). They feed on the pulp of the fruit around the stone, which becomes soft and darker in color.

The yellow traps with glue present a high power of attraction for dipteran species, being useful in establishing the population level of the species *Rhagoletis cerasi* L. and its dynamics, leading to the application of warning treatments at the optimal times (Istrate and Manole, 2019).

The different species of the *Monilinia* pathogen are common pathogens of the stone fruit species (Hrustí´c, J. Et al.,2012, Popa et al., 2012, 2013, Moldovan et al., 2022), producing effects regardless of the culture system and applied treatments. Attacked fruits are often infected by species of the genus *Monilinia* (*Monilinia laxa*, *M. fructigena* and *M. fructicola*) known as a pest often found in Europe (EPPO, 2007, 2020; Usall et al., 2015).

In addition to fruit, the pathogen also infects the aerial parts of host plants with a variety of symptoms, including flower burn, wounding on woody tissues, and fruit rot during the growing season, but also in the post-harvest phase (Florian et al., 2018; Paraschivu et al., 2020).

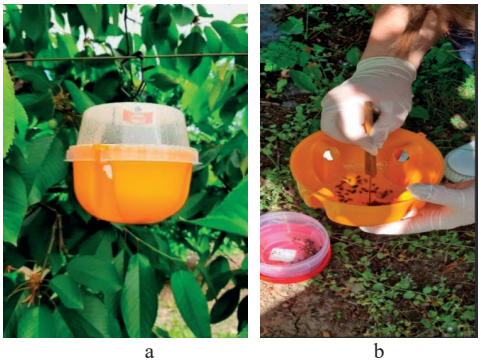
Brown rot of cherry fruits is one of the most important diseases. Injured, dying, or dead tissues of the trees can be infects by *Leucostoma* canker (Cotuna et al., 2020).

In chemically untreated cherry orchards, the frequency of attack can reach up to 100%, and the fruits lose their commercial value (Istrate and Roșca, 2009; Abang et al., 2014). The study of the pathogen *Monilinia laxa* included research on the degree of attack on leaves, shoots, and fruits, which was calculated based on the indicators, frequency (F%) and intensity of attack (I%), while the inspection was initiated in May with continuation in June, with the monitoring of the pest *Rhagoletis cerasi* L. The severity of the disease is largely determined by the weather; the symptoms vary from year to year (Gheorghieș and Geamăn, 2003). Disease severity is expected to increase during wet or rainy weather with mild daytime

temperatures (20-25°C) and cool nights (EPPO, 2020; Chițulescu et al., 2019).
The results of the research were put in line with the microclimate recorded during the determination period. In recent years, due attention has not been paid to the health of the plantations, recording large harvest losses due to the deterioration of the quality of the fruits.

MATERIALS AND METHODS

Research on the appearance and evolution of *Rhagoletis cerasi* L. adults was carried out in the cherry orchard of the University of Agronomic Sciences and Veterinary Medicine of Bucharest, during 2024, made up of a mixture of varieties: Regina, Skeena, Giorgia, Kordia, Ferrovia, Lapins, New Star, Van, Giant Red, Early Red, Rubin, Severin, Istrita, Katalin, Stefan, Ulster, Paul, Sam, Bigareau Burlat, Boambe de Cotnari, Vega, Margo, Germersdorf, Katalin, Rivan, Nimba, Royal Tioga, Pacific Red, Roket, Fisco, Alex, Margonia, Andante, Musatin, Amaris.
In order to monitor the species, before the cherries come into fruition (the second decade of May), yellow Decis Trap traps were placed in the crown of the trees, at a height of 1.5 m and a distance of approximately 10 m between them. The Decis Trap traps were installed on 23.04.2024, 5 traps per row (Figure 1a), a number of 25 traps, in 5 rows.



a b
Figure 1.a) Installation of traps Decis Trap; b) collection of captured insects (foto: Rada Istrate, 2024)

Observations were made daily until the first specimens appeared, then weekly, noting each reading (Figure 1b). On the basis of the obtained data, the graph of the evolution of the pest was drawn up (the beginning of the appearance of the adults and the maximum flight).
The Decis trap, is a trap designed to attract and combat the species *Rhagoletis cerasi* L. It is a trap consisting of a plastic container made up of two parts: the upper part is colorless and transparent, impregnated on the inner surface with the active substance, deltamethrin 0.015 g/trap, and the lower part of the trap is orange and serves to store flies caught and contains a sachet consisting of a strong attractant (ammonium carbonate), specific for *Rhagoletis cerasi* L. The sachet with attractant is dosed so that its elimination is done gradually, providing a duration of protection of 5 months. The assembled trap forms a closed system with 2 cm diameter side holes through which the flies enter. The method of identifying the pathogen *Monilinia laxa* was realized visually by observing the manifestation of symptoms on the plants under the conditions of the year 2024. To the varieties that showed symptoms, the methodology used in plant protection was applied: three branches arranged in different directions and floors, and on each branch, 100 shoots were examined.
For the examination of the fruits, 300 fruits per branch were analyzed, and finally the average was obtained for each variety. The evaluation technique consisted in noting the frequency of the attacked organs (attack incidence, %) and the intensity (attack severity, %) in which the symptoms were manifested. These phytosanitary indicators were used in the evaluation of the behavior of the varieties: A.D. (%) = F (%) x I (%)/100. For the intensity of the diseases, marks were granted on a scale from 0 to 4.
The research results were correlated with the regional temperatures and precipitation recorded during the determination period. (Rungjindamai et al., 2014), so that the attack of the pathogen and the pest can be controlled by combining several cultural practices (cultural hygiene by removing debris, winter treatments applied in winter windows, and performing chemical treatments). During 2024, three chemical treatments were carried out on: 01.02.2024 with Oleomin 80 EW, 1.3% + Faster 10 CE - 0.02% + Oviperon Top - 1.5% + Flipper 497.8 EW - 1.5%; 18.03.2024 with Decis Expert 100 EC - 0.05% + Score 250 EC -

0.02%; 12.04.2024 with Fastac 10 EC - 0.02% + Folicur Solo - 0.1% (Table 1).

Table 1. Treatments carried out in the cherry orchard

Date	The active substance	Products
01.02.2024	Ulei mineral horticol 80%	Oleomin 80 EW - 1.3%
	Cipermetrin 100 g/l	Faster 10 CE - 0.02%
	Ulei de parafină 800 g/l	Oviperon Top - 1.5 %
	Săruri de potasiu ai acizilor graşi C7-C18 79.8 g/l	Flipper 497.8 EW - 1.5%
18.03.2024	Deltametrin 100 g/l	Decis Expert 100 EC - 0.05%
	Difenoconazol 250 g/l	Score 250 EC - 0.02%
12.04.2024	Alfa-cipermetrin 50 g/l	Fastac 10 EC - 0.02%
	Tebuconazol 250 g/l	Folicur Solo 250 EW - 0.1%

RESULTS AND DISCUSSIONS

In order to obtain healthy fruits, it is necessary to know the *Rhagolesti cerasi* L., species population and the dynamics of its appearance as well as its evolution in the crown of the trees during the months of April, May and June of 2024. To monitor the cherry fly, we aimed to analyze the activity of the cherry fly under the influence of climatic factors and chemical treatments.

During 2024, the first appearance in the cherry orchard was in the first decade of May (06.05.2024), where between 0 and 8 captures/trap were recorded at a temperature of 19°C and an atmospheric humidity of 56%. The appearance of the first specimens in the first decade of May was also reported in the Iași region by Chelaru et al. (2021).

The first captures of adults in the Decis Trap traps were recorded in the first decade of May with an average of 1.6 adults/trap. The activity of adults increased significantly, so that in the second decade of May, 110 specimens/25 traps were captured in traps with an average of 4.4 adults/trap (Table 2). On May 12th-13th, the air temperature dropped to almost 14°C due to light precipitation, and the humidity was 78% (Figure 2).

The maximum level regarding the activity of the species *Rhagoletis cerasi* L, was reached in the first decade of June (03.06.2024) with a catch of 61 adults/trap recorded on row 8, located in the middle of the orchard, at a temperature of 25°C and a humidity of 60% (Figure 3).

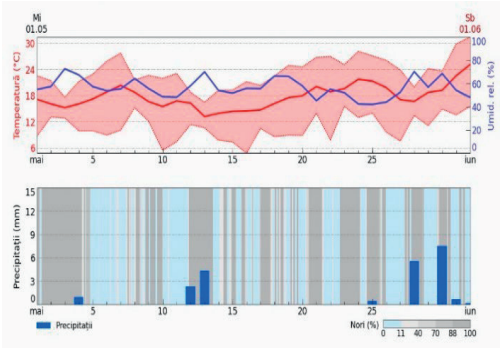


Figura 2. Graph: top) temperatures (red) and relative air humidity (light blue); down) precipitation (mm). May, 2024. Source: https://www.meteoblue.com/ro

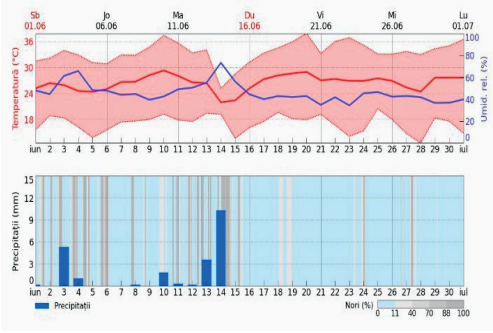


Figura 3. Graph: top) temperatures (red) and relative air humidity (light blue); down) precipitation (mm). June, 2024. Source: https://www.meteoblue.com/ro

In the third decade of May (17.05.2024), due to a colder climate, the activity of adults is lower with an average of 3.98 adults/trap. In some traps no adults were recorded in the Decis Trap traps. As the air temperature increased (22°C), more adults were captured, with a total of 185 specimens/25 traps and an average of 7.4 specimens/trap. The most intensive flight period was recorded on row 8, with an activity of 43 adults/trap, being also located in the middle of the cherry plantation. During the month of May, a total of 433 specimens were captured in the Decis Trap traps.

In June, the flight of the species *Rhagoletis cerasi* L. took place during the period 03-18.06, with a high frequency in the first decade of the month, with a total of 367 captures/25 traps and an average of 14.68 adults/trap (Table 3).

Consistent and significant presence observed in almost all Decis Traps.

Table 2. Evolution of the species *Rhagoletis cerasi* L., during May, 2024

Variants	Trap	6.05.2024	13.05.2024	17.05.2024	24.05.2024	Total catches
R27	C1	0	2	6	5	**13**
	C2	2	5	2	0	**9**
	C3	0	3	0	1	**4**
	C4	1	5	7	6	**19**
	C5	2	6	3	1	**12**
R29	C1	0	0	1	5	**6**
	C2	4	6	2	1	**13**
	C3	0	2	3	3	**8**
	C4	0	3	4	1	**8**
	C5	5	6	13	0	**24**
R31	C1	0	1	1	4	**6**
	C2	0	1	0	3	**4**
	C3	1	5	4	5	**15**
	C4	0	4	5	6	**15**
	C5	5	10	3	9	**27**
R33	C1	3	11	8	24	**46**
	C2	6	12	7	25	**50**
	C3	8	11	14	20	**53**
	C4	2	8	6	43	**59**
	C5	0	3	4	7	**14**
R35	C1	0	0	1	2	**3**
	C2	1	4	0	1	**6**
	C3	0	1	0	2	**3**
	C4	0	1	2	7	**10**
	C5	0	0	2	4	**6**
Total		**40**	**110**	**98**	**185**	**433**

Table 3. Evolution of the species *Rhagoletis cerasi* during June, 2024

Variants	Trap	03.06.2024	12.06.2024	18.06.2024	26.06.2024	Total
R27	C1	6	8	1	0	**15**
	C2	4	1	0	0	**5**
	C3	6	9	0	0	**15**
	C4	43	1	0	0	**44**
	C5	11	0	0	0	**11**
R29	C1	11	8	1	0	**20**
	C2	5	5	0	0	**10**
	C3	6	3	0	0	**9**
	C4	5	1	1	0	**7**
	C5	13	3	1	0	**17**
R31	C1	10	11	0	0	**21**
	C2	7	16	6	0	**29**
	C3	11	0	0	0	**11**
	C4	11	0	2	0	**13**
	C5	17	1	0	0	**18**
R33	C1	12	7	2	0	**21**
	C2	21	5	0	0	**26**
	C3	44	12	0	0	**56**
	C4	61	20	1	0	**82**
	C5	50	12	0	0	**62**
R35	C1	2	1	0	0	**3**
	C2	0	0	0	0	**0**
	C3	0	0	0	0	**0**
	C4	4	1	0	0	**5**
	C5	7	1	0	0	**8**
Total		**367**	**126**	**15**	**0**	**508**

Starting from the second decade, lower temperatures and a humidity of 73% were recorded, which led to a decrease in the activity of the cherry fly in the crown of the trees

(Figure 3). Towards the end of June, a decrease in the number of adults in the traps is observed, so that starting from 26.06.2024, no adults appear in the analyzed samples.

During the year 2024, the species *Rhagoletis cerasi* appeared in the Decis Trap traps starting from the first decade of May, with a number of 8 adults/trap, after which the number of the population increases to 46 adults/trap on 13.05. Amid low temperatures and rainfall, the population drops to 23 adults/trap, then increases towards the end of May to 43 adults/trap. The maximum flight was recorded in the first decade of June with a peak of 61 adults/trap. As the fruits are harvested, the population begins to drop significantly to 20 adults/trap on 12.06, then to 6 adults/trap on 18.06, and after 26.06, no adults appear in Decis Trap traps. It can be seen in figure 4 that as the air temperature is higher, the number of adults caught in the Decis Trap traps also increases (Figure 4).

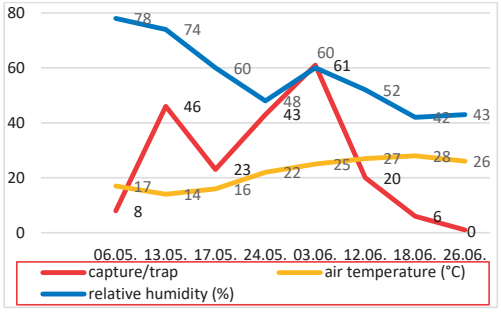


Figure 4. Population dynamics of *Rhagoletis cerasi*, 2024

The relatively small number of adults of *Rhagoletis cerasi* caught in traps was also due to the chemical treatment carried out when the species appeared in the crown of the trees, starting from the second decade of May (12.04.2024), with Fastac 10 EC - 0.02%. The cherry fly population was also kept under control by capturing the insect in Decis Traps. The largest population captured in the Decis Trap traps was recorded in the first decade of June, after which the density of the *Rhagoletis cerasi* species begins to decrease, so that towards the end of the month it is non-existent in the crown of the trees.

The fruits attacked by *Rhagoletis cerasi*, show deeper and darker areas on the outside, with time they become soft, turn brown and rot due to infections with the fungus *Monilinia* laxa (https://www.anfdf.ro/). *Monilinia laxa* is favored by the attack of these insects (Zală et al., 2007).

Among the cherry varieties analyzed, aspects of *Monilinia laxa* attack on inflorescences and shoots were highlighted in the Regina, Giorgia, Van, Boambe de Cotnari, Vega, and Rivan varieties. The first infections appeared at the beginning of May, in the varieties Boambe de Cotnari and Amaris. The attack on young shoots led to wilting, bending of the tip down, and their withering, and the frequency of attack was 10% on shoots in Boambe de Cotnari variety and 15% in Amaris variety. The attack of the pathogen was manifested in these varieties also on the flowers, showing browning and withering, remaining hanging on the shoots. During May, the temperatures recorded, correlated with the relative air humidity between 60-80%, allowed the pathogen *Monilinia laxa* to develop in good conditions on the attacked shoots, ultimately leading to their drying.

The high temperatures in June allowed the infections to spread to the fruits, where the appearance of round brown spots was initially observed. The infection on the fruit bunches settled from their center, expanding outwards. Finally, the fruits showed a soft rot, the flesh browned, and the appearance of specific fruiting was evident (Figure 5).

In June, the varieties where symptoms produced by the pathogen *Monilinia laxa*, anamorph stage *Monilia laxa* (Figure 6) were identified had an incidence of shoot attack between 3.54% in the Van variety and 30.25% in the Rivan variety (Figure 7).

At the fruit level, the attack incidence was a minimum of 6.78% in Regina and a maximum recorded in the Boambe de Cotnari variety, 32.25% (Figure 8).

The intensity of the shoot attack had a minimum in the Vega variety (9.51%) and a maximum of 45.25% in the Rivan variety. The intensity of the fruit attack had values of 10.31% in the Van variety and 53.1% in the Giorgia variety (Figure 9). Manifestations of moniliosis in these varieties during previous years were also highlighted by Zală et al. (2022).



Figure 5. *Monilinia laxa*- fruit attack
(foto: Zala Cristinel Relu, 2024)

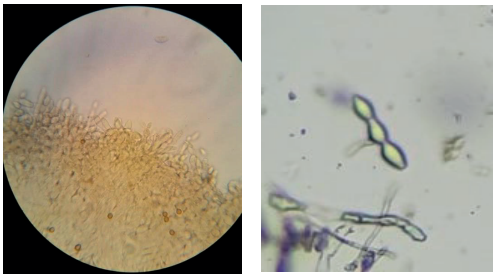


Figure 6. *Monilia laxa* - anamorph stage
of *Monilinia laxa* (foto: M.S. Manole, 2024)

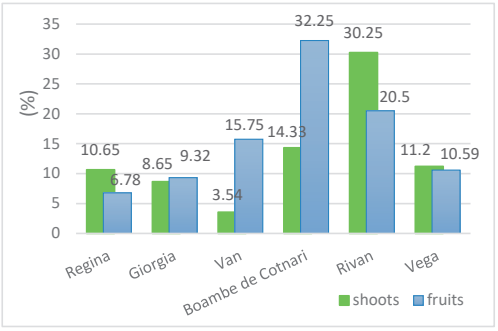


Figure 7. The incidence of attack
by *Monilinia laxa* to shoots and fruits

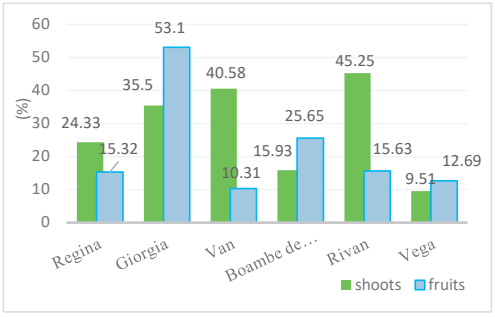


Figure 8. Intensity of attack by *Monilinia laxa*
on shoots and fruits

The values of the phytosanitary indicators led to the calculation of a degree of attack at the shoot level of 1.06% in the Vega variety and 13.68% in the Rivan variety. In fruits, the highest degree of attack was recorded in the Boambe de Cotnari variety, at 8.27% (Figure 9).

Everhart et al. 2011 consider that the locations of attacks on shoots from the previous year may play an important role in the manifestation of attacks in the current year.

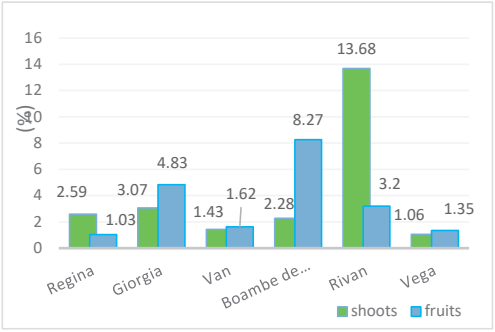


Figure 9. Degree of attack on shoots and fruits,
Monilinia laxa

CONCLUSIONS

In the year 2024, the population of the species *Rhagoletis cerasi* L. appeared in the crown of the trees in the first decade of May, followed by a significant increase in the second decade of May, after which the population decreases against the background of some precipitations recorded on May 12th and 13th.

Decis Traps have proven useful in tree crowns, both for monitoring and controlling *Rhagoletis cerasi*. Depending on the demographics of the population, the optimal time to apply chemical treatments can be determined.

The dynamics of *Rhagoletis cerasi* L. populations in the cherry orchard within the didactic experimental field of USAMV Bucharest highlighted the fact that the appearance of adults took place in the first decade of May, with a maximum flight in the first decade of June, followed by a gradual decrease as follows so that at the end of June there was a lack of adults in the Decis Trap traps.

In the context of climate change, a significant increase in the cherry fly population was found,

being present in the crown of the trees right from the beginning of May.
The observed pathogen appeared on flowers and young shoots at the beginning of May, and in June, the pathogen *Monilinia laxa* was noted in the crown of the trees on ripe cherry fruit.
During the monitoring of the disease attack, we observed an increase in *Monilinia laxa* infections with fruit ripening.
It was also found that where there was a large number of adults captured in traps, the manifestation of rot on cherry fruits was also more intense, knowing that the presence of the cherry fly produces pathways for the pathogen to enter the fruit.

ACKNOWLEDGEMENTS

This article was financed by the Faculty of Agriculture, University of Agronomic Sciences and Veterinary Medicine of Bucharest.
This research activity was carried out with the support of Mr. Daniel Potor, an engineer who is in charge of the Experimental Didactic Field of the Department of Pomiculture within USAMV Bucharest, and with the materials sponsored by Bayer Romania.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

RESULTS OF THE COMPARATIVE RESEARCH ON DISEASES ASSOCIATED WITH INVASIVE NEMATODES IN MAIZE PLANTATIONS UNDER THE CONDITIONS OF THE REPUBLIC OF MOLDOVA

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Abstract

In the Republic of Moldova, maize, also known as corn, is one of the major field crops, possesses strong phenotypic plasticity, is profitable and productive, but every year it is frequently invaded by harmful organisms, which exert a severe parasitic impact. The results of the phytosanitary control realized each year in the corn plantations revealed a considerable diversity of 6 diseases caused by 15 species of phytopathogenic fungi. Besides, the frequency values and the progressive impact were assessed comparatively per phases of vegetation, attacked organs, crop rotation systems and monoculture. The above-mentioned pathogens were also associated with invasive nematode complexes-20 species included in 7 families, belonging to the Tylenchida order, distributed according to the investigated areas, classified into 5 groups of the trophic spectrum. It has been estimated that the highest values of frequency and abundance of the invasive impact are characteristic of the detected endo-ectoparasitic species belonging to the families: Pratylenchidae Hoplolaimidae, Paratylenchidae, Tylenchidae, Heteroderidae, which severely infest the seedlings in the early stages of vegetation.

Key words*: corn, pathogenic fungi, invasive nematodes, phytosanitary control, parasitic impact.*

INTRODUCTION

Corn cultivation in the Republic of Moldova is of universal importance for the primary and secondary finished product used in alimentation, in the livestock sector and various industries (Moraru, 1998; 1999; Starodub, 2015; www.porumbeni.md). It is advantageous due to the high productivity as compared with other cereal crops; it has advanced ecological plasticity; is an efficient precursor for some field crops, absolutely mechanized cultivation techniques, with high rates of organic fertilizers use, minerals and water; various facilities for the caryopsis use and marketing of production and as seed material (Bobeș et al., 1992; Vasilică, 2003; Bîlteanu, 2003; Butnaru, et al., 2004; Pojoga & Mateciuc, 2010; Starodub et al., 2011; Pîrvan, 2019).

These advantages are due to the bioecological adaptation qualities under the influence of abiotic stress factors such as: relative tolerance to drought and heat, mechanical resistance; extended leaf area, mechanisms that ensure efficient water consumption, homeostasis regulation and induction of specific resistance. However, the disadvantages include the attack of a large number of harmful organisms, including communities of parasitic nematodes associated with complex insect pests from the soil, even in the first stages of growth (germination - formation of 3-5 leaves, which cause serious damage to corn annually (Busuioc, 2004; Baldwin et al., 2004; Perry et al., 1999; Siddiqi, 2000; Perry & Moens, 2006).

The phytosanitary biological control, performed periodically on corn plantations is essential for the detection of invasive associations of nematodes and diseases, for the elaboration and application of remedial prognoses to the adjustment of the parasitic impact with the host plant. The successful management of nematode complexes and diseases from soil and terrestrial ones provides for the development of an effective program of integrated protection, which is based on keeping the population of harmful organisms below the economic threshold of damage, to be harmless to the entire

agroecosystem (Dekker, 1972; Andrassay, 1976; Nesterov, 1997; Baicu & Stanescu 1999; Baldwin et al., 2004; Rosca et al., 2000; Berca, 2000; Busuioc, 2004).

The respective investigations were carried out in various areas and sectors of corn and seed production, compared to the researched experimental sectors for breeding and approval conditions of new forms and hybrids within the "Porumbeni" Institute of Phytotechnology. Our purpose is: to carry out phytosanitary monitoring on complexes of invasive nematodes and harmful diseases in corn, comparative with various productive sectors, nurseries, experimental approval soils, with establishing the parasitic impact in the technological management of integrated protection procedures, according to the economic threshold of damage. Based on the mentioned purpose, we set the following research objectives:

- The estimation of the diversity and structure of parasitic nematode complexes associated with harmful diseases and their agroeconomic impact on corn growing.
- Carrying out phytosanitary records to establish the degree of invasive helminthological and pathological impact on corn, comparatively, depending on areas, in productive, experimental and demonstration sectors.

MATERIALS AND METHODS

The investigations were carried out by mutual agreement between the Corn Seminology and Technology laboratories of the "Porumbeni" Institute of Phytotechnology and the Parasitology and Helminthology laboratory, Institute of Zoology, 2022-2024 (Figures 1-A, B, C). The investigations were conducted in the field for the evaluation of breeding of corn seeds and promising hybrids, various productive and seeding sectors with an area of over 600 ha, distributed in experimental plots and homologation lands of newly created hybrids, suitable for cultivation. Breeding forms and productive promising hybrids are grown in three repetitions on plots with an area of 4.9 m^2 and a density of 55-65 thousand plants per ha (30-35 plants on a row). We researched more than 350 approved and promising corn hybrids, 260 parental forms and 60 homozygous lines, used in the breeding process.



A B C

Figure 1. A, B, C. Research and teaching activities, mutual collaboration in the laboratory and in the experimental sector with new hybrids, *"Porumbeni" Institute of Phytotechnology,* 2022-2024

The homologated corn hybrids and promising ones from the Northern area (Pelenia v., Drochia d.), State Station for Field Crop Testing and Homologation were researched comparatively. Seed registration was performed according to the classification scale after the reaction of corn plants to nematode complexes and pest diseases. The corn was monitored periodically, immediately after the germination of caryopsis (10-12 days until the formation of 8-10 mature leaves), with phytosanitary assessments. Over 300 soil samples were taken at a depth of 15-30 cm, as well as samples of plants affected by parasites. The establishment of the helminthological and pathogenic diseases was performed visually in the field, with the help of a magnifying glass (optical degree, 100 MM), and in order to ascertain the criteria of extensiveness and the level of attack, indices of parasitic disease were used, through the values of numerical density (D. n./m^2, plant), frequency (F%), intensity (I%), reflecting the extent of the attack (GA%), reported in m^2, with the analysis of 100 plants/10 samples, by finding the diseases at the level root or plant, using the gradation of 5 points (0 points - no attack, 4 points - serious diseases > 50%). Subsequently, the soil and plant samples taken, were subjected to laboratory analyzes by additional observations, using the binocular magnifier MBS - 10 and the binocular microscope, highlighting the level of phytoparasitic attack, plant diseases were confirmed with the Canon EOS 1000 D camera

(Iurcu et al., 2004; Rosca et al., 2000; Starodub, 2015; Pîrvan, 2019).

The surveys to determine the severity of diseases in corn were carried out according to Iurcu et al., 2004. From 10-20 samples assessed diagonally, by 10 plants each were visually analysed and the values of the assessment parameters were estimated, according to the 5-point grading scale for scoring the level of attack, in which: 0 - there are no symptoms of pathological or parasitic damage; 1 - the affected area is up to 10%; 2 - from 11 to 25%; 3 - from 25% to 50%. 4 < 50% of the plant organ surface is affected (Dekker, 1972; Romașcu, 1973; Andrassay, 1976; Nesterov, 1997; Siddiqi, 2000; Baldwin et al., 2004; Bădărău & Bivol, A. 2007; Bădărău, 2012).

Following field and laboratory analyses, the etiological composition and diversity of pathogens and insect pests of agroeconomic importance were established, the severity of the impact was calculated by using formulas of phytoparasitic indices such as: *numerical density (D), attack frequency (F%), attack intensity (I%)*, estimation scales used to assess the reaction of corn plants to the attack of pathogens and parasitic insects (Iurcu & Lazu, 1996; Rosca et al., 2000; Decramer & Hant, 2006; Bădărău, 2012; Nichișiceva, 2012; Starodub, 2015).

RESULTS AND DISCUSSIONS

In order to determine the level of pathological contamination of corn plants in the experimental sectors of the "Porumbeni" Institute of Phytotechnology, including other productive sectors in the North and Centre zones of the Republic of Moldova for grain and silage production, on areas of over 1200 ha, periodic surveys were carried out to record key diseases during the growing season, germination-full ripening and establishing the etiological composition of the pathogens, the frequency and intensity of the attack (Figure 2 - A, B).



A B

Figure 2. A, B. Phytosanitary surveys conducted to determine the invasive impact and diseases caused by nematode associations and other diseases affecting corn plants, "Porumbeni" Institute of Phytotechnology (Pașcani v., Criuleni d., 2023)

Table 1 reflects the etiological composition and the level of impact of pathogens that trigger various dangerous diseases in corn plants, detected in various experimental and productive plantations with various assessments of frequency and intensity of the attack. As a result of surveys conducted on corn diseases, in the growing seasons of 2020-2024 6 key diseases were identified, caused by 19 pathogens specialized in this traditional crop, regardless of climate change, the implementation of new hybrids, innovative technologies, maintenance procedures and productivity. We can estimate, based on phytosanitary surveys, that the most severe were the fungal phytopathogenic agents that cause various forms of rot (dry or wet, ascending as the plants grow, in all plant organs). Under the conditions of the Republic of Moldova, we have detected pathogens of the genus *Fusarium,* the species *F. moniliforme, F. graminiarum, F. culmorum*, *F. oxysporum, F. gibbosum, F. sambucinum,* followed by corn smut - *Ustilago zeae,* (DS), Corda; and head smut of corn *Sorosporium reilianum,* Mc.Alpine. Significant damage was caused by the species *Nigrospora oryzae, Diplodioza (maydis), Giberela (zea), Aspergillus flavus.*

Table 1. The etiological composition of pathogens and diseases in corn plants detected during the growing season and the intensity of pathological impact, 2022-2024, Phytotechnology Institute, Republic of Moldova

Disease / pathogen	Disease frequency (F %)		Attack intensity (I %)	Affected organs
	Germination-panicle formation phase	Flowering-ripening phase		
1. Head smut of maize - *Sporisorium reilianum*, Mc.Alpine;		**5-25**	**3-15**	Generative organs
2. Corn smut - *Ustilago maydis,* (DS),Corda;	**3-17**	**25-30**	**10-17**	Generative and vegetative organs
3. Various forms of dry rot root affecting the roots, stem and ears, caused by species of the genus *Fusarium* :				Fusarium rot lesions were detected on all plant organs in all development stages
F. graminiarum Schw,				
F. moniliforme, Schw.,			**20-60**	
F. oxysporum Schle,	**15-70**	**30-90**		
F. gibbosum App.,				
F. culmorum Sac.,				
F. avenaceum Sac.,				Stems, ears in ripening-storage phase.
F. roseum Linc.,				
4. Nigrospora rot - *Nigrospora oryzae* (Berk.et Br.),Petch.;	**10-20**	**15-45**	**10-25**	
5. Corn leaf blight - *Helminthosporium turcicum*, Pass.;	**7-15**	**20-30**	**10-20**	Elliptical brown spots on leaves and husks, premature rotting of leaves
6. Dry rots and blight caused by the associative complex of saprophytic pathogenic fungi -*Diplodia zeae* (Schwabe) Lev., *Gibberella zeae* Schw, *Rhizopus nigricans* Ehrenb., *Mucur mucedo* L. *Aspergillus flavus* Link. *Trihotecium roseum* Pers. *Rizoctonia bataticola* Taubenh., *Alternaria* sp. etc...	**3-7**	**7-15**	**5-10**	It gradually attacks all organs in the maturation and ripening phases, especially causing falling and breakage of stems and cobs.



A B C

Figure 3. A - Cob affected by corn smut; B - Panicle affected by smut; C - Cob affected by rot

All these pathogenic species also cause various forms of rot, first of all in young roots at the beginning of growing season, and then they affect the stem and the cobs; once the growing season ends, when cobs are harvested and moved into storage warehouses where they are also dangerous by causing rots in harvested caryopses (Figure 3 - A, B, C).

In recent years, during the corn flowering phase, because of the favorable periods caused by the torrential rains in June-July, in some hybrids and in some sectors, helmintosporiosis frequently occurred locally and to an advanced degree on adult leaves, especially if there are sorghum plants in the vicinity (which are more sensitive to this pathogen). The pathogen is *Helmintosporium turcicum*, which is also estimated in the sectors of the "Porumbeni" Institute of Phytotechnology, in the nurseries of parental forms and creation of new lines, serious diseases of 20-40% were reported in certain areas with typical pathological signs of this disease. In the productive sectors, the intensity of attack was reduced by 5-12%, reported during the ear formation phase, specifically in the terrace areas of the Nistru River, in the districts of Anenii Noi, Criuleni and Dubasari. Therefore, the following diseases specific to corn are common under the conditions of the Republic of Moldova (Figure 4 - A, B, C), causing the greatest damage, such as: dry and wet rot, blight, dusty and common forms of smut,

helminthosporiosis, nigrosporiosis and diplodiosis (Bădărău & Bivol, 2007; 2021; Bădărău, 2012; Bivol & Bădărău, 2022), accompanied by the association of harmful insects specific to these plants such as: species of wireworms and false wireworms, corn weevils, species of beetles, corn borer, species of aphids etc.



A B C

Figure 4. A - experiemntal sector under phytosanitary investigation at "Al. Ciubotaru" NBGI; B, C - Corn plants affected by fusariosis, in the harvesting phase (photo by Bivol A., Ciobanu V., Criuleni d., September 2023)

These harmful organisms detected annually in corn plants are under the permanent scrutiny at the "Porumbeni" Institute of Phytotechnology and are mandatorily included in the periodic phytosanitary record programs, short and long-term forecasts included in the corn breeding studies, both in terms of quality and quantity, and in analyses of the evolution of plant resistance to the detected harmful organisms (Pojoga & Mateciuc, 2010; Pîrvan, 2019).

As compared with the productive sectors, the corn breeding and hybridization nurseries have an invariable location and include all the breeding processes needed to creating valuable traits such as: precocity, productivity, quality and resistance to diseases and pests. This valuable cereal crop is researched annually under conditions of progressive monoculture, which favors intensive accumulations of phytopathogenic infections and harmful insects in these sectors, which allows the creation of challenging backgrounds, significant for revealing forms that are resistant to the most important diseases and pests.

These associations of helminthic invasive agents and harmful diseases on corn plants are scrutinized by researchers, who frequently carry out investigations and phytosanitary records, which are included in research programs in the field of phytotechnology and plant breeding aimed at creating new forms, inbred lines, new corn hybrids, with resilience and tolerance to environmental stress factors. According to bibliographic sources, the corn crops suffer damage annually; over 200 species of harmful organisms, of which 20-25 species are very dangerous, invasive and, under unstable climate conditions, cause significant diseases in corn plants, their severity depending on the area, precocity, seed material, hybrid, environmental factors (Bobeș et al., 1992; Iurcu & Lazu, 1996 ; Iurcu et al., 2004; Badarau & Bivol, 2007; Bivol & Badarau, 2022). Phytosanitary records on corn areas and sectors were carried out periodically, in late April - May - June - July - August. The months of May-June were characterized by periodic abundant rainfall, slight increase in temperature, which determined the abundance of reproductive potential of hatching of invasive larvae, with aggressive infestation capabilities in plants, increasing the degree of parasitic helminthological impact, already in the phases of formation of leaves, stems until the formation of panicles and cobs. In these months, late spring - summer the phytohelminthosis symptoms remain accentuated, more advanced and extensive by the dynamics of visual disorders of yellowing, low number of mature leaves, poorly developed, dwarf plants with roots severely affected by necrosis and specific rot, caused by helminths. The frequency and intensity values of parasitic helminthic impact advance from 3% to 25%, which indicates the damage of more advanced corn plantations in the early stages (germination, 5-8 adult fungi), according to the sensitivity of plants to frequent frosts and instability of factors medium.

Table 2. The comparative indices estimating the helminth parasitic impact on corn crops, in average values, by investigated areas and districts, May-August, 2024

Areas and districts investigated	May 25, 2-3 leaves			June 20, 5-10 leaves			20 July, flowering - pollination			August 25, caryopsis formation and ripening		
	D (100 g sol)	F (%)	I (%)	D (100 g sol)	F (%)	I (%)	D (100 g sol)	F (%)	I (%)	D (100 g sol)	F (%)	I (%)
North, Drochia d., Pelinia v.	15-30	10-15	3-10	40-180	15-25	10-15	30-150	12-17	10-12	120-250	15-30	12-18
Center, Criuleni d.	50-80	5-10	3-7	60-180	10-17	7-12	50-180	3-10	3-7	140-280	10-20	7-12
South-East, Căușeni d., Grigorievca d.	40-100	7-20	10-15	70-150	15-28	12-15	20-90	15-20	10-12	80-180	15-25	10-15
South, Ceadîr-Lunga, t. Svetloe v.	60-120	5-15	7-10	130-240	12-20	8-12	40-130	10-15	7-10	40-90	10-20	8-13

The estimated values in Table 2, characterize the comparative indices of the parasitic impact degree, by numerical density (D ex./100 g soil), frequency of diseases (F %), intensity of attack (I %), reflecting the extent of the attack, compared to the number of soil samples analyzed and plants per m^2, compared per sectors and areas investigated. The numerical density (D) of the parasitic nematode complexes is by 10-25% higher in middle-late June than in May, respectively, and the frequency and intensity of attack is more advanced in the summer, being facilitated by favorable environmental conditions, high prolificacy and available food ration (Table 2). These investigations are important for establishing a qualitative and quantitative diagnosis of the parasitic helmitological impact, for the elaboration of forecasts in the application of integrated protection measures, in the capitalization of corn plantations with various precocity and cultivation areas.

Successful management of harmful organisms is ensured by the application of a permanent phytosanitary control program and integrated protection procedures, which includes a number of components, including control methods by reducing the number of harmful organisms and the parasitic impact. The approach in the phytosanitary practices of integrated management of harmful organisms is based on keeping the population of the harmful organism below the economic damage threshold, with periodic control records. Another important aspect in completing the helminthological investigations in corn is the study of establishing the frequency and structure of the parasitic nematode complexes noticed in the researched corn plantations. For the first time in the Republic of Moldova, such research on corn was performed with the aim of establishing the parasitic impact indices of specialized parasitic nematodes adapted to corn and the taxonomic abundance in investigated areas, with the distribution of these harmful organisms according to the spectrum of trophic specialization, reflected in the Table 3. The results of taxonomic analyzes showed the presence of 21 species from 8 families with diverse trophic specialization (endo-ecto-semi-endoparasite), migratory and sedentary. We detected the presence of species from the families *Hoplolaimidae, Paratylenchidae, Telotylenchidae, Criconematidae, Neotylenchidae, Tylenchidae*, in the late spring-summer periods in various forms and biological stages, extracted both from the soil and from the roots of plants. All species were classified according to parasitic trophic specialization in 5 groups, with the predominance of endo- and ecto-parasitic forms of absorbent bristles, according to the way of adaptation, growth phase, biotope, environmental factors.

Table 3. Results of the taxonomic analysis of parasitic phytonematode communities detected in corn, comparatively per investigated areas, 2020-2024

Taxonomic name of the species detected	Phytoparasitic trophic specialization	North area	Center area	South - East area	South area
I. Pratylenchidae fam.:					
1. *P. subpenetrans*	Migrants - endoparasites	++	++	++	++
2. *P. nanus*		++	++	++	++
3. *P. curvatus*		+	++	++	+
4. *P. hamatus*		+	+	+	+
II.. Paratylenchidae fam:					
5. *P. cuvitatus*	Migrants - ectoparasites	+	+	++	++
6. *P. aciculus*		-	+	+	++
7. *P. nanus*		++	++	+	+
8. *P. tenicaudatus*		+	+	+	-
III. Hoplolaimidae fam.:					
9. *Helicotylenchus digonicus*	Semi-endoparasites,	++	++	++	+
10. *H. dihistera*	nutrients absorbing bristles	++	++	+	++
11. *Rotylenchus agnetis*		++	+	++	++
12. *R. incultus*		+	++	++	+
IV. Fam. Telotylenchidae:	Ectoparasites of absorbent				
13. *Amplimerlinius dubius*	bristles	-	+	+	+
14. *Merlinius brevidens*		+	+	+	+
15. *Bitylenchus parvus*		+	+	+	-
V. Criconematidae fam.:	Ectoparasites of absorbent				
16. *Mezocriconema xenoplax*	bristles	+	+	+	+
17. *Xenocriconemella macrodora*		-	+	+	+
VI. Neotylenchidae fam.:	Ectoparasites				
18. *Psilenchus aestuarius*		-	+	+	-
19. *P. aberans*		+	+	+	+
VII. Tylenchidae fam.:	Endoparasitic-migratory	++	++	+	+
20. *Tylechus filiformis*					
VIII. Heteroderidae fam.:	Cyst forming	+	+	++	++
21. *H. avenae*					
Total: 8 families 21 species	**5 trophic-parasitic specialization groups**	**21**	**26**	**27**	**25**

CONCLUSIONS

The successful management of the diseases estimated in the conducted studies ensures the implementation of a permanent phytosanitary control program and integrated prevention and protection procedures, which include a series of ways to regulate the number and reduce the impact of invasive pathogens. The results of the helminthological and pathogenic phytosanitary investigations conducted in 2020-2024 on corn crops, estimate the indices of diversity and structure of invasive nematode communities and disease from the soil, with comparative establishment of seasonal parasitic impact, vegetation phases, cultivation technologies and ecological areas.

As a result of the comparative phytopathological investigations carried out on

corn crops, during the selected vegetation period, the presence of 34 species of harmful organisms and 19 pathogens that cause specific diseases with severe impact on plants, which annually cause various types of smut, root, stem and ear rot, and are characterized by different frequency and intensity, causing great damage to corn crops depending on the area, the environmental factors and the resistance capacities of the hybrids used, was detected under the agroclimatic conditions of the Republic of Moldova.

Following the surveys and helminthological analyzes performed on corn, the degree of infestation was established, by estimating the comparative indices of numerical density (D), in variable values on areas of 15-280 ex./100 g soil, with the prevalence of indices of 25-30% more abundant by during May-June, comparative to July-August.

The structure of the populations of parasitic phytonematodes in corn crops was determined by helminthological analyses with morphological and taxonomic aspects, resulting in a total number of 21 species included in 8 families and 2 orders, distributed according to the investigated areas and classified according to

the spectrum of trophic specialization into 5 groups, with the predominance of species in the South-East area (27 species), followed by the Central area (26 species), the South area (17 species), and the North area (25 species).

ACKNOWLEDGEMENTS

The research was carried out with the support of the institutional project – State Program: 20.80009.7007.12 F and the Subprogram 010701, within MSU, as well as the subprogram 010102, MSU "Al. Ciubotaru" NBGI.

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www.porumbeni.md

Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

EFFECT OF LEGUME-BARLEY INTERCROPPING ON POPULATION DYNAMICS OF CEREAL APHIDS IN DIFFERENT BARLEY VARIETIES

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Abstract

Intercropping increases plant diversity in the agroecosystem and thus supports regulatory mechanisms for pest populations. Aphids are economically important pests of barley and represent a significant threat to cereal grain production. The research was carried out in 2021 and 2023 under field conditions in Vrazhdebna, Sofia (42°70'76.1"N, 23°43'73.1"E). The intercropping system consisted of different varieties of winter feed and malting barley and legumes (chickpea and vetch) sown in rows of strips. This study was designed to investigate the effect of legume-barley intercropping on cereal aphid populations and barley production. The data obtained showed that the combination of barley and legumes resulted in a reduction in aphid numbers of up to 31.7%. The analysis revealed no statistically significant differences in aphid attack between different cultivars of winter feed and malting barley grown in mixtures with legumes. The LER values for economic yield indicate that this cropping system is conducive to barley production and effective in pest management within the agroecosystem.

Key words*: intercropping, cereal aphids, barley.*

INTRODUCTION

Barley (*Hordeum vulgare* L.) is a major cereal crop grown throughout the world. The ability of barley to adapt to different climates and its wide range of uses underline its importance in the world's agriculture and food industries. Barley can grow successfully in temperate climates and is highly valued for its versatility. Approximately 70% of the world's barley production is used as animal feed, while the remaining 30% is used as a source of fermentable material in various products. Barley constitutes an integral component of the Bulgarian agricultural sector, being employed in diverse industrial applications, including animal fodder, brewing, distilling, and numerous other sectors (Gramatikov et al., 2004).

Intensive agriculture is characterised by short crop rotations, inadequate spatial isolation, and a paucity of biodiversity in agrocenoses. This combination of factors results in an elevated risk of the proliferation of significant pests (Tanchyk et al., 2024).

Aphids (Aphididae: Hemiptera) are a group of insects that have been identified as a significant problem for numerous agricultural crops worldwide. There has been a notable increase in their population in recent years, and they have become a persistent pest (Singh & Singh, 2021). Aphids are one of the most important pests and one of the major plant health problems of barley in Europe (Namara et al., 2024). Infestation with aphids in winter barley can lead to significant yield losses through direct feeding damage and the transmission of plant viruses, particularly Barley Yellow Dwarf Virus (BYDV) (Lau et al., 2021). Aphids extract essential nutrients by feeding on the phloem sap of barley plants. This weakens the plant and can reduce yields by up to 10% (https://www.agric.wa.gov.au/). In addition to direct damage, aphids are vectors for barley yellow dwarf virus (BYDV), a virus that poses a significant threat to barley crops. Infected plants often show stunted growth and yellowing of leaves, leading to significant yield losses. In some trials, BYDV has been associated with yield losses of up to 80% in barley (Grauby et al., 2022; https://cropscience.bayer.co.uk /agronomy-id/pest-and-slugs/).

During the initial growth stages of cereals, these crops exhibit an increased vulnerability to yellow dwarf disease. Most of the damage to winter cereals is attributable to infections sustained during the autumn season (Fabre et al., 2003). The bird-cherry aphid (*Rhopalosiphum padi* (L.)), for instance, has been observed to

overwinter on volunteer cereals and grasses, thus rendering early sown autumn cereals particularly vulnerable to BYDV infection (White et al., 2023). Climate change, and in particular global warming, may result in the prolonged presence of aphids in cereal fields, thereby increasing the risk of infection with BYDV (Van den Eynde et al., 2020). This suggests a potential increase in aphid damage in the future (Grauby et al., 2022). The most important pests of barley in Bulgaria are Corn leaf aphid *Rhopalosiphum maidis* (Fitch, 1856), Wheat aphid *Schizaphis graminum* (Rondani, 1852) and English grain aphid *Sitobion avenae* (Fabricius, 1775) (Maneva, 2010). The management of aphids is largely contingent upon the application of insecticides. Despite the effectiveness and rapid action of insecticides, their extensive utilisation gives rise to a multitude of deleterious consequences. These include the advent of insecticide resistance in aphids and deleterious effects on beneficial and other non-target organisms (Müller, 2024). Furthermore, there is a risk to human health and environmental pollution. These dangers should not be ignored. Therefore, it is necessary to implement alternative and environmentally friendly pest control practices. Changes in control strategies have led to increased interest in long-term control techniques that have the potential to diminish reliance on insecticides in the context of sustainable agriculture. An important tool in sustainable aphid management is to create an environment that supports and encourages natural enemy population. Intercropping is a practice that has been utilised for this purpose. There is some evidence in the literature that cereal aphid populations decrease in intercropping systems compared to monoculture (Sarwar, 2011; Duan et al., 2022; Grauby et al., 2022). Furthermore, intercropping has the potential to offer valuable food and spatial resources to the agroecosystem for the enhancement of beneficial insects (Liu et al., 2017).

Intercropping has been demonstrated to increase plant diversity within the agroecosystem, thereby supporting regulatory mechanisms for pest populations by providing new levels of protection against aphids and, consequently, against viral diseases (Grauby et al., 2022; Saied et al., 2024).

Despite the existence of literature pertaining to the efficacy of combining cereals with other crops for aphid control, there is a paucity of research focusing on the role of intraspecific diversity, specifically the combination of various cultivars of a given cereal species. In this context, the aim of the present study was to investigate the potential effect of combining barley and legumes in mixed cropping of various barley cultivars on the abundance of cereal aphid. The study will also determine the role of strip intercropping as a barley protection practice and its application under real production conditions.

MATERIALS AND METHODS

The investigations were carried out in the field at the Training field of the University of Forestry in Sofia (42°70'76.1"N, 23°43'73.1"E) during the vegetation period 2021-2023. The region's climate is classified as temperate continental, with an annual mean precipitation of 620.8 mm and an average of 500 to 1.750 hours of sunshine. The mean annual temperature is 12.02 °C. The soil in the area is categorised as slightly stony Fluvisols, as defined by the classification system of the Food and Agriculture Organization (FAO).

Field experiment design

The trial was conducted in a combination of intraspecific (mixture of barley cultivars) and interspecific (barley-legumes intercropping) cropping practices. The cropping system included barley (*Hordeum vulgare* L.) 6 winter feed barley cultivars (LG Zebra, Rafaela, Zanzibar, California, LG Callista and Veslets) and 6 winter malting barley cultivars (Casanova, Hemus, Emon, Tangra, Zagorets and Obzor), vetch (*Vicia sativa* L., cv. Obrasets 666) and chickpea (*Cicer arietinum* L., cv. Plovdiv 8). The barley crop was sown in strips in the autumn, with different cultivars next to each other. The sowing depth was 5 cm, with an inter-row distance of 13 cm and an intra-row distance of 6 cm. In the spring, vetch and chickpea were sown successively between the strips, with a sowing depth of 5 cm, a row spacing of 20 cm, and an inter-row distance of 20 cm. No plant protection measures were applied, except for the application of herbicide after sowing the crops. The total area of the experimental block was 600

square metres, with each experimental plot comprising 18 rows of crops, each measuring 5 metres in length.

The experiment was meticulously designed using a complete randomised block design (RCBD), encompassing 4 distinct variants and 3 replicates. The following variants were subjected to testing: WFB: winter feed barley 18 rows: 3 rows for each cultivar; WMB: winter malting barley: 18 rows; 3 rows for each cultivar; WFB + LVC: 18 rows /6 rows winter feed barley /1 row for each cultivar/ + 3 row vetch и 3 row chickpea +6 rows winter feed barley/1 row for each cultivar/; WMB + LVC: 6 rows /6 rows winter malting barley /1 row for each cultivar / + 3 row vetch и 3 row chickpea + 6 rows winter malting barley/1 row for each cultivar/.

Sampling of cereal aphids

Aphid dynamics in each variant and cultivar were monitored by means of direct visual observations. The start of the assessment coincided with the appearance of the first individuals on the barley plants or at growth stage BBCH 49 and continued until the aphid populations were completely exhausted or at BBCH 87. The quantity of cereal aphids was evaluated on pre-tagged streams, which had been randomly selected. Individuals on the 20 streams for each cultivar were enumerated at 10-day intervals, from which an average value aphid individuals per stream was calculated. The number of aphids of the established species was summarised, as well as the number of winged (alatae) and wingless (apterae) forms. The abundance of aphids over the entire observation period was estimated as the sum of the mean number of individuals in each variant or cultivar for the whole research period.

The effect of intercropping was assessed using a quantitative parameter, the land equivalent ratio (LER), which compares the economic yield obtained by combining the specified crops with the yield obtained by growing the same crops in monoculture (Chapagain & Riseman, 2014).

Statistical analysis

The data were subjected to analysis of variance (ANOVA) and mean differences (3 replicates × 3 years, n = 9) were determined by Duncan's multiple range test ($P \leq 0.05$) using the statistical software package Statistica 13.0.

RESULTS AND DISCUSSIONS

The most important species of cereal aphids: *Rhopalosiphum padi* (Linnaeus, 1758), *Rhopalosiphum maidis* (Fitch, 1856), *Schizaphis graminum* (Rondani, 1852), and *Sitobion avenae* (Fabricius, 1775) (Hemiptera: Aphididae) were identified on the barley crop. Only a few specimens of *Myzus (Nectarosiphon) persicae* Sulzer, 1776 and *Metopolophium dirhodum* (Walker 1849) have been recorded. Despite the variability in the percentage of the four species (*R. padi*, *R. maidis*, *S. graminum* and *S. avenae*) observed over the course of the study, the most frequently encountered species were *S. avenae* (73.2%) and *S. graminum* (18.4%). The species *R. padi* (5.1%) and *R. maidis* (3.3%) were found in limited quantities. The data were obtained from the mean number of aphids per plant, as recorded during the BBCH growth stage 49÷87, with the mean calculated over the three-year study period. The highest number of aphids was observed during the BBCH growth stage 65÷71. The mean total aphid number for the three sampling seasons is demonstrated in Figure 1.

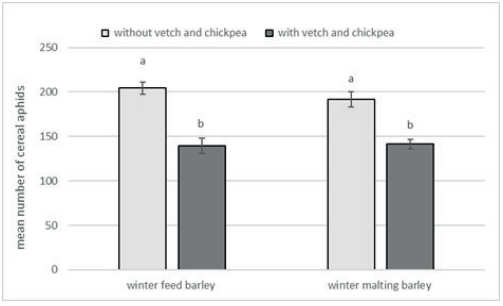


Figure 1. Figure 1. Mean total number of cereal aphids during the period 2021-2023

(mean ± SE; different letters within the same variant (bars with the same color) indicate statistically significant differences, Duncan's multiple range test, $P \leq 0.05$)

The abundance of cereal aphids exhibited variation amongst the different variants, with the highest recorded numbers observed in winter feed barley (204.3 aphid individuals) and winter malting barley (191.7 aphid individuals). Conversely, the mixed chickpea and vetch variants exhibited a reduced aphid abundance, with 139.1 individuals/stream recorded in the WFB variant and 141.3 aphid individuals in the

WMB variant. The most significant reported decrease in numbers by 31.7% was in the winter feed barley and legume variant, in comparison to the winter feed barley cultivars cultivated without legumes. The total number of aphids in combinations of all cultivars of winter feed and winter malting barley with legumes was statistically proven, as compared to the total number of aphids when grown without legumes (Figure 1).

The results of the analysis of the attack data for each cultivar are shown in Figures 2 and 3. When growing winter feed barley cultivars without legumes, cv. LG Zebra had the lowest number of aphids (15.3 individuals/stream), and cv. Veslets the highest (53.6 individuals/stream) followed by cv. Zanzibar (49.1 individuals/stream) and cv. Rafaela (37.4 individuals/stream), while the cultivars California and LG Callista were intermediate with numbers of 25.4 and 23.5, respectively. The statistical analysis confirmed the differences found between the varieties (Fig. 2).

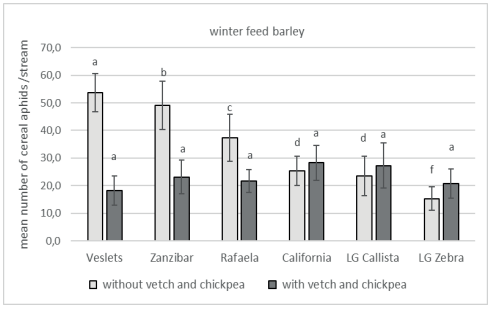


Figure 2. Mean number of cereal aphids on various winter feed barley cultivars

(mean ± SE; different letters within the same variant (bars with the same color) indicate statistically significant differences, Duncan's multiple range test, $P \leq 0.05$)

When winter malting barley cultivars were cultivated without legumes, similar data were reported. The statistically highest aphid abundance was found in the cv. Emon (50.9 aphid individuals/leaf and ear) and cv. Tangra (42.2 individuals/stream). The lowest abundance was observed in the cv. Obzor (12.7 individuals/stream) and cv. Casanova (21.4 individuals/stream), while the cv. Zagorets and cv. Hemus occupied an intermediate position with abundances of 36.4 and 28.1 individuals/stream, respectively (Figure 3).

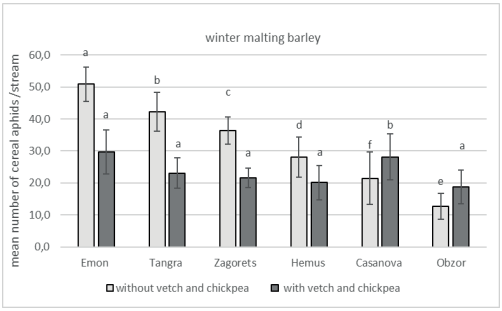


Figure 3. Mean number of cereal aphids on various winter malting barley cultivars

(mean ± SE; different letters within the same variant (bars with the same color) indicate statistically significant differences, Duncan's multiple range test, $P \leq 0.05$)

Data on the number of aphids in different cultivars of winter feed barley grown together with legumes (chickpea and vetch) showed that there were no statistically proven differences between the varieties when compared with the same varieties grown without legumes. It should be noted that among the cultivars of winter malting barley grown with legumes, the differences are not significant, but the cultivars that were most affected (Emon and Tangra) when grown without legumes then occupy an intermediate position when grown with legumes. However, the cultivars most severely attacked in the absence of legumes presented an intermediate response when grown in conjunction with legumes.

The reported significant reduction in numbers of aphids in the mixed crop of the cultivars with legumes does not allow us to reject the hypothesis that aphid populations do not decrease when grown without legumes. This is because in our study the varieties were not grown independently, and comparisons cannot be made. However, the results regarding the reported differences between cultivars, or lack thereof, in the two cropping practices could be explained by the fact that the effect of intraspecific diversification on pest regulation depends on biotic pressures (Huang et al., 2012). Similar results were documented by Mansion-Vaquié et al. (2019), who ascribed the absence of an impact on aphid abundance when combining distinct wheat varieties to the minimal biotic pressures. During the period of our studies, the highest aphid abundance recorded was (53.6 individuals/stream),

indicating a low level of attack (Schnelle, 1995). The results show that the abundance of aphids differed in the two cropping systems. A significant interactive effect was observed in co-cropping with legumes, which resulted in a reduction in the number of aphids. Based on information from the literature (Sarwar, 2011; Grettenberger &Tooker, 2016) and our research, it should be assumed that the number of aphids in cereal crops depends on annual variations and specific environmental conditions, microclimatic and stress factors, such as prolonged drought, and the growing seasons during which we conducted the surveys were characterised by below-normal rainfall. Therefore, research over a longer period is needed. The expected reduction in aphid populations by mixing barley and legumes was confirmed by our results. This was true for both feed and malting barley.

The extant literature pertaining to the mechanisms of this form of intercropping is diverse. According to Mansion-Vaquié et al. (2019), the reduction in aphid numbers during the co-cropping of wheat and white clover is associated not only with the diversity of the aboveground cover, but also with differences in the nitrogen content of the plants. The nitrogen content is an important parameter for determining the quality of the plant as a host for aphids (Zehnder & Hunter, 2008). The authors further report that during this period, the nitrogen content in the wheat grain was lower. Another factor that must be considered is the way the crops that enter the intercropping system are arranged. (Lopes et al., 2016). Since the findings of our study, we can conclude that the use of strip cropping systems for mixed cultivation, which resulted in an increase in species diversity in the agroecosystem, led to a reduction in the number of aphids. Liu et al. (2020) also reported a few successful examples of aphid control using strip intercropping, where wheat-pea intercropping reduced populations of pea aphid *Acyrthosiphon pisum* (Hemiptera: Aphididae) and S. avenae compared to pure stands. A reduction in the infestation of wheat by aphids such as *S. graminum* and *S. avenae* was observed when wheat was intercropped with strips of lucerne (Saeed et al., 2013). Intercropping systems increase crop diversity in agroecosystems, have an impact on the abundance of herbivorous insects and their natural enemies, and can be an important tool in the reduction of cereal aphid abundance (Arshad et al., 2018).

The Land Equivalent Ratio (LER) was calculated to determine the effect of competition between the species used in the intercropping system. The total LER was found to be 2.01 in the winter malting barley and legume variant and 2.07 in the winter feed barley variant. The LER values indicated good crop development (Dhima et al., 2007).

CONCLUSIONS

The present study has concluded that the intercropping system of barley and the legumes chickpea and vetch influences the abundance of cereal aphids by reducing their numbers. The reduced aphid damage prevents yield losses in barley without affecting the yield of the legumes. This finding indicates that the proposed cropping system is a viable approach for both production and aphid management within cereal and legume agroecosystems.

However, extensive research is necessary, including the identification of the factors that led to the specific reactions of the studied varieties in the system of intraspecific diversification and the monitoring of meteorological parameters in specific agricultural regions. This will assist in the identification of suitable pest control strategies within the framework of integrated plant protection.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

RESEARCH ON THE IMPACT OF NITROGEN AND PHOSPHORUS FERTILIZERS ON WINTER WHEAT YIELD AND QUALITY UNDER THE PEDOCLIMATIC CONDITIONS OF CENTRAL MOLDOVA

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Abstract

Winter wheat is a key agricultural crop that responds positively to fertilizers under the soil and climatic conditions of our country. However, factors influencing fertilizer efficiency and dosing challenges necessitate the implementation of long-term studies to investigate the complex interactions among plant, soil, fertilizer, and climate, as well as their impact on crop yield. This paper presents findings from a long-term study on the use of chemical fertilizers (NP) in winter wheat, carried out at the Agricultural Research and Development Station Secuieni, Romania (26°5` E; 46°5` N) during the period 2020-2023. The yields obtained from applying chemical fertilizers varied depending on the applied dose. On average, the studied factors had a considerable influence on wheat yield, resulting in significant variability in yield, ranging from 4886 kg ha^{-1} (unfertilized, N_0P_0) to 7117 kg ha^{-1} ($N_{80}P_{160}$ active substance). The interaction between phosphorus and nitrogen on wheat seed yield demonstrated a significant impact, contributing to increases of up to 21.7% and 16.1%, respectively, compared to the control.

Key words*: climate, nitrogen, phosphorus, plant, quality indicators, winter wheat, yield.*

INTRODUCTION

Straw cereals, especially wheat (*Triticum aestivum* L.), are the most widely cultivated plant in the world, grown in over 100 countries, and are a prime commercial source (Voinea & Ilie, 2023).

Winter wheat is one of the most important crops in the world of particular economic importance, with bread made from wheat flour being the essential food, also ensuring about 20% of total calories consumed (Chiriță et al., 2023). Wheat is an important source of energy and protein for humans all over the world. As wheat flours have the unique properties to form a strong and elastic dough, the majority of wheat is consumed in form of baked goods and a high baking quality is required (Rossmann et al., 2020).

Fertilization is a basic technological element of modern agriculture. The complex process of absorption of nutrients is dependent on a number of biotic and abiotic factors and the interaction between them (Șimon et al., 2022). Agrochemical optimization of the soil-plant system is the essential alternative to achieve high-quality, stable yields and is achieved through the rational and balanced application of fertilizers, differentiated and integrated, with sustainability according to crop requirements and effective soil input (Rusu, 2021).

It is well known that nitrogen significantly influences the growth and development of plants. Knowledge of the physiological mechanisms that contribute to the absorption and use of nitrogen is particularly important to increase the efficiency of the use of this chemical element (Agapie et al., 2021).

Wheat is a crop that efficiently uses chemical fertilizers with nitrogen and phosphorus, nitrogen being the chemical element that causes higher yield increases. The superiority of wheat

yield achieved by using nitrogen is natural, considering that nitrogen represents the "pivot of fertilization", the decisive factor in increasing wheat yields, and phosphorus has a smaller effect (Ceclan et al., 2024).

During the vegetation period, the accumulation of nitrates in the soil does not occur at the same time as the plants' requirements, and for this reason, the application of chemical fertilizers is required to ensure the necessary nutrients for plant growth. Wheat needs nitrogen in the spring for the completion of germination and for vegetative growth, or during this time it often happens that the soil has a small amount of nitrogen (Dodocioiu & Buzatu, 2024).

Efficient utilisation of phosphorus (P) is paramount for crop production, resource sustainability, and food security. For responsible management of P fertilisers across varying fertilisers supply scenarios in wheat cultivation, it is imperative to systematically assess the impact of soil characteristics, crop varieties, and crop rotation practices on P efficiency (Yan et al., 2024).

The efficient capture and utilisation of fertiliser nitrogen (N) by cereals has implications for crop growth, grain yield, farm profits, the environment and human nutrition (Duncan et al., 2018).

Rapid innovation in modern agriculture has revealed that optimized P fertilizer placement allows to reduce P fertilizer input, thereby effectively increasing fertilizer use efficiency and crop yield (Du et al., 2020).

The combinations containing mineral N showed a significant improvement in yield stability in which N + P and N + K are almost identical. In all fertilisation variants, the additional supply of manure stabilised the wheat yields. The mineral supply of N + P + K with additional manure provided the best yield stability and lowest agronomic risk for yield failure (Macholdt et al., 2018).

In the modern agriculture, the importance of using chemical fertilizers is undeniable. In the structure of chemical fertilizers, those with nitrogen occupy the main place due to their contribution in determining the yield increase, as well as due to the weight with which they participate in the applied fertilization formulas (Dumitrașcu et al., 2003; Mihăilă et al., 1996; Petcu et al., 2003).

MATERIALS AND METHODS

The experience was placed from 2020 to 2023 at the Agricultural Research and Development Station Secuieni (A.R.D.S. Secuieni) on a typical cambic faeoziom (chernozem) soil with medium texture. The experience was conducted on a typical cambic chernozem soil type, middle texture, acid: pH_{H2O} - 5.98, characterized as: supplied in phosphorus (77.6 ppm P_{AL}), Ca (13.6 mEq/100 g soil Ca) and Mg (1.8 mEq/100 g soil Mg), middle supplied in active humus (1.88%) and nitrogen (16.2 ppm $N\text{-}NO_3$) and poorly supplied in potassium (124.6 ppm K_2O) (Pochișcanu et al., 2017).

The experience was established using a subdivided plot design. The main plots were assigned to different phosphorus doses (0, 40, 80, 120, and 160 kg active substance P_2O_5/ha), while the subplots were allocated to varying nitrogen doses (0, 40, 80, 120, and 160 kg active substance N/ha). Fertilization was carried out using ammonium nitrate and superphosphate, with the amount of fertilizer applied corresponding to the designated phosphorus and nitrogen doses in each treatment. In the field, in this case, the cultivation technology specific to the conditions in Central of Moldavia was used, and the data obtained was interpreted statistically according to the method of variance analysis (Leonte & Simioniuc, 2018). The experimental setup followed a three-year crop rotation system comprising wheat, corn, and beans.

The analysis of temperature and precipitation trends during the winter wheat vegetation period in the Secuieni area indicates a shift towards increasingly hot and dry conditions. These meteorological extremes have the potential to cause significant agricultural losses.

The temperatures recorded in the three agricultural years, compared to the multiannual average, indicate clear warming in most months of the year, except April and May, where the temperatures were lower than the multiannual average by 0.4°C and 0.2°C, respectively.

This warming trend is more pronounced in the winter and early spring months, but summers are also becoming progressively warmer (Table 1). Between October and July, the average temperature was 9.1°C, which is 1.9°C higher than the multiannual average of 7.3°C over the past 60 years.

Table 1. Temperature registrated at A.R.D.S. Secuieni during winter wheat vegetation period

Month	2020/ 2021	2021/ 2022	2022/ 2023	Monthly average	Multiannual average 60 years	Dev.
October	10.8	8	11.5	10.1	9.2	0.9
November	7.8	5.6	5	6.1	3.6	2.5
December	1.8	-0.2	0.8	0.8	-1.5	2.3
January	-0.6	-0.1	2.4	0.6	-3.7	4.3
February	3.4	2.6	1	2.3	-1.9	4.2
March	6.2	2.7	6	5.0	2.8	2.2
April	10	9.5	8.1	9.2	9.6	-0.4
May	13.9	16.3	15.4	15.2	15.4	-0.2
June	20	20.7	19.9	20.2	18.9	1.3
July	20.9	22.2	22.6	21.9	20.4	1.5
Monthly average	9.4	8.7	9.3	9.1	7.3	1.9

In terms of precipitation over the entire growing season of the wheat crop, deviations from the multiannual average were varied, with their distribution being extremely uneven across the plant's growth and development phases. The smallest deviations, compared to the multi-year average for the same periods, were 4 mm in December, while the largest deviations were recorded in July, with 33.1 mm (Table 2). From the sowing of the winter wheat to harvest, the crop experienced a significant precipitation deficit of 179.5 mm, compared to the multiannual average for the same period.

Table 2. Precipitation registrated at A.R.D.S. Secuieni during winter wheat vegetation period

Month	2020/ 2021	2021/ 2022	2022/ 2023	Monthly average	Multiannual average 60 years	Dev.
October	33	3	19.8	18.6	36.9	-18.3
November	14.6	10.8	41.7	22.4	27.7	-5.3
December	6.2	39	19	21.4	25.4	-4.0
January	2	5.4	13.2	6.9	19.6	-12.7
February	16	4.6	12.6	11.1	19.2	-8.1
March	10.2	0.8	7.6	6.2	26.3	-20.1
April	1.2	38.4	38.4	26.0	44.9	-18.9
May	69.6	20.8	21	37.1	64.3	-27.2
June	72.6	56.6	29.8	53.0	84.7	-31.7
July	39	35.2	68.2	47.5	80.6	-33.1
Monthly average	264.4	214.6	271.3	250.1	429.6	-179.5

RESULTS AND DISCUSSIONS

Climatic conditions during the growing season are the main factor that determines crop yield, especially in areas where the only source of water is from rainfall and groundwater and even the extreme temperatures can significantly reduce crop yield.

Phosphorus fertilizer application (after the averages of five graduations of nitrogen fertilizers) resulted in yields ranging from 7145-8647 kg·ha^{-1} in 2020-2021, 4460-6326 kg·ha^{-1} in 2021-2022, and 4940-5285 kg·ha^{-1} in 2022-2023. On average, the influence of phosphorus on yields ranged from 5515 kg·ha^{-1} in the variant unfertilized, to a maximum of 6714 kg·ha^{-1} in the variant where the phosphorus dose applied was 160 kg of active substance.

Yield increases from phosphorus fertilizer application (2020-2023 average) from 550 kg·ha^{-1} (10%) at the P_{40} dose to 1199 kg·ha^{-1} (22%) at the P_{160} dose (Table 3). The yields obtained, compared to the control variant, were superior and were statistically confirmed and interpreted as highly significant.

Table 3. The influence of phosphorus fertilizers on winter wheat yield

Nitrogen dose (kg/ha)	Yield kg· ha^{-1}				Relative yield (%)	Dif.	Sem.
	2020/ 2021	2021/ 2022	2022/ 2023	Average yield			
P_0	7145	4460	4940	5515	100	Mt.	Ct.
P_{40}	7636	5448	5112	6065	110	550	***
P_{80}	7801	5766	5151	6239	113	724	***
P_{120}	8647	5983	5285	6638	120	1123	***
P_{160}	8539	6326	5276	6714	122	1199	***
LD 5%	46	87	99	77			
LD 1%	61	120	140	107			
LD 0.1%	79	166	166	145			

Nitrogen fertilizer application (after the averages of five graduations of phosphorus fertilizers) resulted in yields ranging from 6827-8668 kg·ha^{-1} in 2020-2021, 5337-6119 kg·ha^{-1} in 2021-2022, and 4604-5418 kg·ha^{-1} in 2022-2023. On average, the influence of nitrogen on yields ranged from 5589 kg·ha^{-1} in the variant with no fertilizer application, to a maximum of 6715kg·ha^{-1} in the variant where the nitrogen dose applied was 120 kg of active substance. Yield increases from nitrogen fertilizer application (2020-2023 average) were 417 kg·ha^{-1} (7%) at the N_{40} dose and 1126 kg·ha^{-1} (20%) at the N_{120} dose (Table 4). The yields obtained, by applying different doses of nitrogen, compared to the control variant, were superior and were statistically confirmed and interpreted as highly significant.

Table 4. The influence of nitrogen fertilizers on winter wheat yield

Phosphor dose (kg/ha)	Yield kg·ha^{-1}				Relative yield (%)	Dif.	Sem.
	2020/ 2021	2021/ 2022	2022/ 2023	Average yield			
N_0	6827	5337	4604	5589	100	Mt.	Ct.
N_{40}	7524	5389	5105	6006	107	417	***
N_{80}	8142	5679	5302	6374	114	785	***
N_{120}	8607	6119	5418	6715	120	1126	***
N_{160}	8668	5459	5335	6487	116	898	***
DL 5%	75	102	46	77			
DL 1%	99	135	61	107			
DL 0.1%	128	174	79	145			

In the first year of experimentation, 2020-2021, yields varied significantly, ranging from 6133 kg·ha^{-1} in the control variant (unfertilized) to a maximum of 9429 kg·ha^{-1} in the variant with an $N_{120}P_{120}$ fertilizer dose, indicating a high efficiency of nitrogen and phosphorus fertilization. In the variants where phosphorus was not applied, increasing the nitrogen dose from N_0 to N_{160} led to a 28.6% yield increase on winter wheat, from 6133 kg·ha^{-1} to 7887 kg·ha^{-1}.

In the second year of testing, yields ranged from 3993 kg·ha^{-1} (unfertilized) to a maximum of 6822 kg·ha^{-1}, achieved in the variant where the applied fertilizer dose was $N_{80}P_{160}$.

Regardless of the nitrogen and phosphorus doses applied to winter wheat, all fertilized variants recorded higher yields compared to the control variant, which received no fertilization. This emphasizes the positive impact of fertilization on plant growth and development compared to the unfertilized variant.

The lowest yield in the final year of testing was recorded in the control variant (4531 kg · ha^{-1}), while the highest yield (5601 kg · ha^{-1}) was achieved in the variant where the applied fertilizer dose was $N_{120}P_{120}$.

During the period 2020-2023, winter wheat yields showed variations due to the combination of fertilizers used, the dosage applied, and weather conditions. Thus, over the three years of testing on winter wheat, yields for the unfertilized variant (N_0P_0) ranged from 3993 to 6133 kg · ha^{-1}, with an average of 4886 kg · ha^{-1}. Compared to this, the average yield increases on winter wheat, resulting from fertilizer application ranged between 4% and 46%, representing 207 to 2231 kg·ha^{-1}.

It was observed that nitrogen fertilizers resulted in higher yield increases in all the years studied. Thus, on the P_0 agrofund, nitrogen fertilizers applied at doses of N_{40}-N_{160} led to yield increases on winter wheat of 4-22%, representing 207-1082 kg·ha^{-1}; on the P_{40} agrofund, increases ranged from 25% to 30%, representing 1206 - 1467 kg·ha^{-1}; on the P_{80} agrofund, increases of 25-26% were recorded, representing 1229-1293 kg·ha^{-1}; on the P_{120} agrofund, increases ranged from 32% to 43%, representing 1541-2114 kg·ha^{-1}; and on the P_{160} agrofund, increases of 35-42% were observed, representing 1690-2052 kg·ha^{-1} (Table 5).

Compared to the control variant (no fertilizer), regardless of the fertilizer dose applied, the yields on winter wheat were higher and were interpreted as highly significant.

Table 5. The influence of nitrogen and phosphorus fertilizers on winter wheat yield during the period 2020-2023

Applied dose	Yield kg·ha^{-1}				Relative yield	Dif.	Semnif.
	2020/2021	2021/2022	2022/2023	Average yield			
N_0P_0	6133	3993	4531	4886	100		***
$N_{40}P_0$	6593	4003	4682	5093	104	207	***
$N_{80}P_0$	7339	4233	5077	5550	114	664	***
$N_{120}P_0$	7772	5348	5113	6078	124	1192	***
$N_{160}P_0$	7887	4720	5296	5968	122	1082	***
N_0P_{40}	6540	4659	4598	5266	108	380	***
$N_{40}P_{40}$	7252	6014	5009	6092	125	1206	***
$N_{80}P_{40}$	7551	5362	5160	6024	123	1138	***
$N_{120}P_{40}$	8322	6156	5300	6593	135	1707	***
$N_{160}P_{40}$	8515	5050	5494	6353	130	1467	***
N_0P_{80}	6604	6229	4659	5831	119	945	***
$N_{40}P_{80}$	7522	5673	5149	6115	125	1229	***
$N_{80}P_{80}$	7944	5932	5314	6397	131	1511	***
$N_{120}P_{80}$	8470	5978	5586	6678	137	1792	***
$N_{160}P_{80}$	8464	5020	5054	6179	126	1293	***
N_0P_{120}	7318	5375	4730	5808	119	922	***
$N_{40}P_{120}$	8154	5849	5278	6427	132	1541	***
$N_{80}P_{120}$	8919	6045	5397	6787	139	1901	***
$N_{120}P_{120}$	9429	6299	5601	7110	146	2224	***
$N_{160}P_{120}$	9416	6168	5417	7000	143	2114	***
N_0P_{160}	7539	6428	4500	6156	126	1270	***
$N_{40}P_{160}$	9096	5225	5406	6576	135	1690	***
$N_{80}P_{160}$	8958	6822	5570	7117	146	2231	***
$N_{120}P_{160}$	9039	6816	5490	7115	146	2229	***
$N_{160}P_{160}$	9061	6339	5415	6938	142	2052	***
5%	103	201	180	161			
1%	137	267	241	215			
0,1%	176	304	317	266			

Regarding the protein content in winter wheat, it ranged from 9% in the unfertilized variant to a maximum of 14.8% in the variant where the applied dose was $N_{160}P_{80}$. The gluten content varied within a wide range, from 16.9% in the unfertilized (control) variant to a maximum of 30.3% in the variant with the applied dose of $N_{160}P_{80}$ (Figure 1).

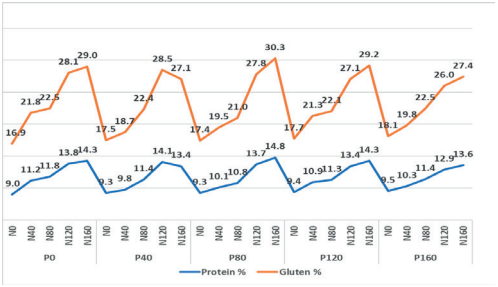


Figure 1. Protein content (%) and gluten content (%) according to the fertilizer dose applied

The starch content on winter wheat reached a maximum value of 71.6% in the control variant, but the application of fertilizers resulted in a

progressive decrease in starch content as the applied dose increased, with a minimum of 67.4% observed at the $N_{160}P_{80}$ dose. Regarding kernel hardness, the lowest values, 19.8%, were observed in the control variant. However, as the fertilizer doses increased, a significant increase in kernel hardness was noted, reaching a maximum of 64.3% at the $N_{160}P_{80}$ dose (Figure 2).

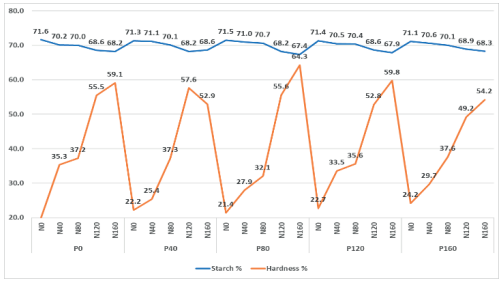


Figure 2. Starch content (%) and wheat kernel hardness (%) according to the fertilizer dose applied

CONCLUSIONS

These results highlight the significant impact of different fertilization levels on winter wheat yield, demonstrating that a higher phosphorus dose contributes to substantial yield increases.

On the P_0 agrofoundation, applying different nitrogen doses (40-160 active substances) led to 4-22% yield increases, equivalent to 207-1082 kg·ha^{-1}. On the P_{40} agrofoundation, the increase ranged from 25% to 30%, totaling 1206-1467 kg·ha^{-1}. For the P_{80} agrofoundation, yields rose by 25-26%, corresponding to 1229-1293 kg·ha^{-1}. The P_{120} agrofoundation recorded increases of 32-43%, amounting to 1541-2114 kg·ha^{-1}. Finally, on the P_{160} agrofoundation, 35% to 42% increases were recorded, equivalent to 1690-2052 kg·ha^{-1}.

ACKNOWLEDGEMENTS

This research work was carried out with the support of the Ministry of Agriculture and Rural Development through the ADER 2023-2026 program from Project no. 18.1.1/27.07.2023 Development of solutions for soil health restoration while maintaining environmental sustainability by harnessing the fertilizing potential of organic fertilizers.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

RESEARCH ON MICROBIOTA ASSOCIATED WITH SUNFLOWER SEEDS

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Abstract

The aim of our research has been to detect and identify the microbiota associated with sunflower seeds. Biological material was represented by seeds samples from romanian hybrids Suria CL and Demetra and from foreign hybrids ES Belfis CLP, LG5077, QC Bravo, P64LE 99, P64LE 162 and P64LE 280. Pathogens from colonies developed after six days were identified, and their incidence was calculated for both untreated seeds and treated seeds with Lumisena 200 FS. In untreated seeds samples fungi from the genera Alternaria, Aspergillus, and Rhizopus were detected as well as bacterial colonies. Alternaria spp. recorded the highest incidence on ES Belfis CLP hybrid (50%), followed by Demetra (30%). Colonies of Aspergillus spp. were detected with an incidence of 33.3% for P64LE 162 hybrid. On treated seeds, Alternaria fungi recorded 20% incidence for Demetra hybrid, and 3.3% for P64LE99 hybrid, while Penicillium spp. showed an incidence of 3.3% for Demetra hybrid. Therefore, the antifungal effect of the tested product has been observed.

Key words*: sunflower, hybrid, seeds, microbiota.*

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the most important oilseeds crops globally, being extensively cultivated in numerous countries, including Ukraine, the Russian Federation, Argentina, China, Romania, Turkey, Bulgaria, France, Hungary, Tanzania, Kazakhstan, Moldova, the United States of America, Spain, South Africa, Serbia, Italy, Uganda, and India. Romania plays a significant role in the international market for this crop due to its considerable production (Chiurciu et al., 2023). Sunflowers are grown primarily for their seeds, which contain 40-50% oil and 17-20% protein (Haj Sghaier et al., 2023).

Seeds harbor diverse microbial groups which play a significant role in maintaining seed vigor, enhancing germination and modulating plant growth (Vannier et al., 2018; Kumar et al., 2024).

The microbial community associated with seeds depends on plant species, seed development, geographical locations, and the presence of other plant pathogens (Houlden et al., 2008; Nelson, 2018).

Pathogens transmitted through seeds pose a significant problem in sunflower cultivation, leading to reduced seed viability and germination as well as seedling vigour, poor stand of the crop in the field and low yields (Afzal et al., 2010). Additionally, their presence affects the biochemical composition of the seeds, including the content of proteins, carbohydrates, and lipids, thus influencing their quality (Mukhtar, 2009).

Among the microorganisms that interact with seeds during storage, fungi play a dominant role in reducing their quality and longevity (Ivan & Cristea, 2015; Cristea et al., 2009).

Studies conducted by Asim et al. (2008) have highlighted the presence of fungi such as *Aspergillus niger, A. flavus, Alternaria zinniae, Cladosporium oxysporum, Curvularia* sp., *Drechslera* sp., *Macrophomina phaseolina, Penicillium* sp., *Phoma* sp., and *Rhizopus* sp. Also, other studies highlight the presence of fungal isolates belonging to the genera

Alternaria, *Aspergillus*, *Cladosporium*, *Mucor*, *Rhizopus*, *Trichoderma* and *Penicillium* associated to sunflower seed microbiota (Levitskaya et al., 2023; Selemani, 2025).
Detection of seed borne pathogens is an important step of integrated pest management strategies in sunflower crops (Guan et al., 2020). Thus, our research focused on detection and identification of the microbiota community associated with sunflower seeds (Dudoiu, 2016).

MATERIALS AND METHODS

The study was carried out on sunflower hybrids cultivated in Zimnicea, Teleorman County, with the aim of identifying pathogens present on seeds. The biological material was represented by seeds from sunflower hybrids EDS Belfis CLP, LG5077, QC Bravo, P64LE 99, P64LE 162, P64LE 280, Demetra and, Suria.
For pathogens isolation and identification, the seeds were incubated on PDA (Potato Dextrose Agar) culture medium at 24°C.
Four experimental variants were tested: untreated seeds, treated seeds with Lumisena 200 FS (200 g/l oxithiapiprolin), seeds washed with distilled water and seeds disinfected with ethanol (immersion for 2 minutes in 70% ethanol, rinsed with distilled water). Seeds treated with water and ethanol have been dried. Each variant was conducted on three replicates (10 seeds/plate/replicate) and the test was repeated twice.

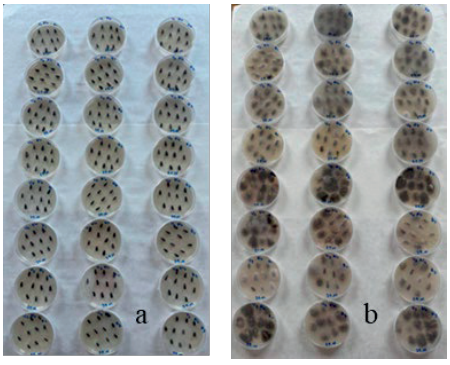


Figure 1. Setting up the experiment: a) day 1; b) day 7

The incidence of isolated colonies was determined by observations made at 3 and 7 days. Macroscopic observations of colonies were followed by light microscopy (Zeiss Primo Star microscope). Results are presented as fungal colonies frequency or incidence (%).

RESULTS AND DISCUSSIONS

The data presented in Table 1 shows the microbiota detected on sunflower tested seeds, the identified colonies belonging to the genera *Alternaria, Penicillium, Rhizopus* and *Aspergillus.*
Our study showed that in Lumisena treated seeds variants (LT) isolates of *Alternaria* spp. were present only in two hybrids, EDS Belfis CLP and P64LE99, and *Rhizopus* spp. occurred in LG5077. *Aspergillus* spp. and *Penicilium* spp. have not been detected (Table 1).
In untreated seeds variants (NT) isolates belonging to genera *Alternaria, Aspergillus* and *Rhizophus* were present in all tested hybrids. Colonies of *Penicilium* spp. have been not detected.
In the ethanol-treated seeds variants (ET), *Alternaria* spp. and *Aspergillus* spp. isolates were present in all hybrids. *Rhizopus* colonies were not present in Suria and QC Bravo and *Penicilium* colonies were present in QC Bravo and P64LE99 hybrids.
In distilled water treated seeds variants (WT), isolates of *Alternaria* spp. as well as those of *Aspergillus* spp. occurred in all hybrids. Colonies of *Aspergillus* were not detected on seeds of P64LE280 hybrid but in this variant *Penicilium* spp. isolates have been recorded. *Rhizophus* spp. colonies occurred in all hybrids, with one exception, Demetra.
The morphology of some colonies is presented in Figure 2.
Microscopic identification of isolates is shown in Figure 3.
The incidence of pathogens is shown in Table 2.
As can be seen, in the Lumisena 200 FS treated seeds isolates of *Alternaria* spp. were present in EDS Belfis CLS (20% incidence) and P64LE99 (3.3% incidence). No colonies of *Aspergillus* spp. have been detected. Isolates of *Penicillium* spp. had an incidence of 3.3% in the hybrid EDS Belfis CLS.
In the untreated seeds variants isolates of *Alternaria* spp. had the highest incidence in

Demetra hybrid (50%) and the lowest incidence in LG5077 hybrid (16.6%).
Isolates of *Aspergillus* spp. recorded the highest incidence in QC Bravo hybrid (33.3%), followed by EDS Belfis CLP and P64LE99 (3.33%). No colonies belonging to *Aspergillus* species were recorded in Demetra, LG5077 and P64LE162 hybrids.

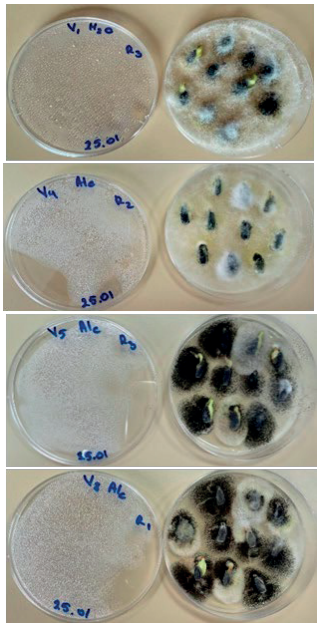


Figure 2. Fungal colonies developed on sunflowers seeds (after 7 days of incubation)

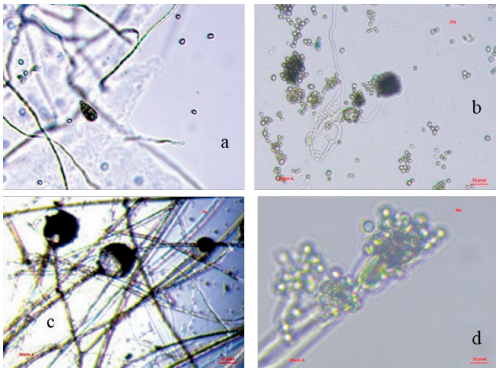


Figure 3. Microscopic identification of detected isolates: a. *Alternaria* spp. - conidia; b. *Aspergillus* spp. - conidia; c. *Rhizopus* spp. - sporangia; d. *Penicillium* spp. - conidiophore and conidia

In the ethanol-treated variants isolates of *Alternaria* spp. have been recorded with an incidence of 50% in Demetra and P64LE99 hybrids. A high incidence of *Aspergillus* spp. isolates has been recorded in EDS Belfis CLP (70%) and P64LE162 (73%) hybrids.
Low incidence values (3.3%) were recorded for *Penicillium* spp. isolates in P64LE99 and QC Bravo and P64LE280 hybrids.
In the water treated seeds variants have been recorded a high incidence of *Alternaria* spp. isolates (50% in LG5077) and *Aspergillus* spp. isolates (83% in QC Bravo). Colonies of *Penicillium* spp. were identified only in P64LE280 hybrid with 3.3% incidence.
The microbiota of the sunflower seeds have been represented by isolates belonging to genera of *Alternaria, Penicillium, Rhizopus* and *Aspergillus.* Our results are consistent with those reported in other studies. Thus, on sunflower seeds, different fungal species belonging to the genera *Alternaria*, *Aspergillus*, *Cladosporium*, *Curvularia*, *Drechslera*, *Fusarium*, *Penicillium* and *Phomopsis* are reported (Mukhtar, 2009). Among them, the common species are *Alternaria alternata*, *A. helianthi*, *Fusarium oxysporum*, *F. solani*, *Penicillium expansum*, *P. brevicompactum*, *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *A. terreus*, *Rhizopus stolonifer*, *Mucor hiemalis*, *Phomopsis macdonaldii* (Abdullah & Al-Mosawi, 2010).
The major seed borne pathogenic fungi recorded in sunflower were *Alternaria alternata, A. helianthi*, *Rhizoctonia bataticola*, *Macrophomina phaseolina* and *Fusarium oxysporum*. Also, saprophytic fungi as *Aspegillus niger, A. flavus,* and *R. stolonifer* were reported (Patil et al., 2018).
The presence of fungal species as *Alternaria alternata, A. helianthi, Aspergillus flavus, A. fumigatus, A. niger, Curvularia lunata, Drechslera tetramera*, *Fusarium solani, F. moniliforme*, *Macrophomina phaseolina*, *Mucor mucedo, Penicillium* spp. and *Rhizopus* spp. on seeds from different sunflower cultivars was found to reduce seed germination by 10-20% and seedling mortality by 10-12% (Afzal et al., 2010).

Table 1. Detected microbiota in sunflower seeds

Var.	Isolates															
	Alternaria spp.				*Aspergillus* spp.				*Rhizopus* spp.				*Penicillium* spp.			
	LT	NT	ET	WT	LT	NT	ET	WT	LT	NT	ET	WT	LT	NT	ET	WT
V1	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-	-
V2	-	+	+	+	-	+	+	+	+	+	-	+	-	-	-	-
V3	-	+	+	+	-	-	+	+	+	+	+	-	-	-	-	-
V4	-	+	+	+	-	-	+	+	-	+	+	+	-	-	-	-
V5	-	+	+	+	-	+	+	+	+	+	-	+	-	-	+	-
V6	+	+	+	+	-	+	+	+	+	+	+	+	-	-	+	-
V7	-	+	+	+	-	+	+	-	+	+	+	+	-	-	-	+
V8	-	+	+	+	-	-	+	+	+	+	+	+	-	-	-	-

V1 - Hybrid EDS Belfis CLP; V2 - Hybrid Suria; V3 - Hybrid Demetra; V4 - LG5077; V5 - Hybrid QC Bravo; V6 - Hybrid P64LE99; V7 - Hybrid P64LE280; V8 - Hybrid P64LE162; LT - Lumisena 200 FS treatment; NT - untreated seeds; ET - ethanol treatment; WT - Water treatment.

Table 2. Incidence of fungal colonies on sunflower seeds after 7 days of incubation

Var.	Isolates incidence (%)															
	Alternaria spp.				*Aspergillus* spp.				*Rhizopus* spp.				*Penicillium* spp.			
	LT	NT	ET	WT	LT	NT	ET	WT	LT	NT	ET	WT	LT	NT	ET	WT
V1	20	30	20	23	-	3.3	70	57	TC	TC	TC	TC	3.3	-	-	-
V2	-	30	40	17	-	6.6	60	70	PC	TC	-	TC	-	-	-	-
V3	-	50	50	17	-	-	50	73	PC	TC	PC	-	-	-	-	-
V4	-	16.6	30	50	-	-	50	23	-	TC	TC	TC	-	-	-	-
V5	-	30	33.3	17	-	33.3	53	83	TC	TC	-	TC	-	-	3.3	-
V6	3.3	23.3	50	37	-	23.3	33.3	50	TC	TC	TC	TC	-	-	3.3	-
V7	-	20	47	23	-	3.3	17	-	TC	TC	PC	TC	-	-	-	3.3
V8	-	20	20	36	-	-	73	60	PC	TC	PC	PC	-	-	-	-

V1 - Hybrid EDS Belfis CLP; V2 - Hybrid Suria; V3- Hybrid Demetra; V4 - LG5077; V5 - Hybrid QC Bravo; V6 - Hybrid P64LE99; V7 - Hybrid P64LE280; V8 - Hybrid P64LE162; LT - Lumisena 200 FS treatment; NT - untreated seeds; ET - Ethanol treatment; WT - Water treatment; TC - total colonization in Petri plates; PC - partial colonization in Petri plates.

CONCLUSIONS

The microbiota of the sunflower seeds have been represented by isolates belonging to genera of *Alternaria, Penicillium, Rhizopus* and *Aspergillus*.

The biological material was represented by seeds from sunflower hybrids EDS Belfis CLP, LG5077, QC Bravo, P64LE 99, P64LE 162, P64LE 280, Demetra and, Suria.

The highest fungal diversity was recorded on untreated seeds. Thus, on these variants, the highest incidence was recorded for *Alternaria* spp. isolates (50%) on Demetra seeds followed by *Aspergillus* spp. isolates (33.3%) on QC Bravo seeds.

Lumisena 200 FS treatment reduced seeds fungal contamination with *Alternaria* spp. isolates compared to untreated seeds. Thus, in EDS Belfis CLP hybrid the incidence of *Alternaria* colonies was reduced from 30% at 20% and in in P64LE99 hybrid from 23.3% to 3.3%. No isolates of *Alternaria* spp. have been detected in the other tested variants.

In ethanol-treated seeds isolates of *Alternaria* spp. and *Aspergillus* spp. have been recorded, with high incidence values in all variants (20-50% for *Alternaria* isolates and 17-73% for *Aspergillus* isolates).

In water-treated seeds variants, high incidence of *Alternaria* spp. isolates (20-50%) and *Aspergillus* spp. isolates (23-83%) have been recorded.

ACKNOWLEDGEMENTS

This research work was carried out within the Doctoral School of Engineering and Management of Plant and Animal Resources, under the careful guidance of the coordinators.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

CORRELATION OF SUNFLOWER YIELD WITH HEAD YIELDING ELEMENTS ACCORDING TO ROW SPACING AND PLANT DENSITY

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Abstract

Sunflower grain yield is positively correlated with head yielding elements (head diameter, the number of grains per head, the weight of grains per head and the 1000-grain weight - TGW), but the correlations of grain yield are stronger with some yielding elements and lower with some others. The present paper aims to identify the particular correlation of sunflower grain yield with head yielding elements according to row spacing and plant density. The analysed data were obtained in the research performed in 2019, 2020, and 2021 in field experiments under rainfed conditions located in four locations in Romania (one in East, one in South-East, and two in South Romania). The experimental factors were the following: Factor A – row spacing, with 3 graduations: a1 - 70 cm; a2 - 60 cm; a3 - 50 cm; Factor B – plant density, with 3 graduations: b1 - 50,000 plants/ha; b2 - 60,000 plants/ha; b3 - 70,000 plants/ha; Factor C – sunflower hybrid, with 4 graduations: c1 - KWS Acer; c2 - NK Neoma; c3 - P64LE25; c4 - Subaro. In the performed research, generally, the coefficient of correlation of sunflower grain yield with the head yielding elements decreases with row spacing decrease and increases with plant density increase. The highest correlation coefficient of grain yield was registered with grain weight per head and TGW regardless of row spacing and plant density. But regardless of plant density, as the row spacing decreases, it decreases the value of the correlation coefficient of grain yield with grain weight per head and increases the value of the correlation coefficient of grain yield with TGW.

Key words*: sunflower, correlation coefficient, yield, yielding elements, row spacing, plant density.*

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is an important oilseed crop in the world, but mostly in Europe, especially in the Black Sea Basin. It offers a wide adaptability, suitability to mechanization, low labour needs, and high oil and protein contents (Canavar et al., 2010).

Sunflower yield is a complex character which depends on several component yield characters (Manivannan et al., 2005). So, like in the case of other crops, sunflower yield is dependent of yield components which have interrelation among them and affect the seed yield directly or indirectly (Gorgjieva et al., 2015).

The yield and the yield components of the head are specific to each sunflower hybrid, but they are influenced by the different growing factors, such as environmental factors (e.g. soil and climatic conditions) and technological factors (e.g. row spacing and plant population) (Ion et al., 2015). It is of great importance to know the relationships between yield contributing characters (Clapco et al., 2019).

Correlation studies in sunflower revealed that grain yield is positively associated with yield components (Gouri Shankar et al., 2006). Generally, the grain yield at sunflower is positively correlated with head yielding elements such as head diameter, the number of grains per head, the weight of grains per head and the 1000-grain weight (Alia et al., 2022; Baraiya et al., 2018; Bran, 2022; Clapco et al., 2019; Ionescu et al., 2021; Jalil et al., 2014; Kalukhe et al., 2010; Kaya et al., 2007; Neelima et al., 2012; Pandya et al., 2015; Radic et al., 2013; Sanju et al., 2018; Singh et al., 2018; Sri et al., 2025; Tyag & Khan, 2013; Varalakshmi et al., 2019; Yasin & Singh, 2018). But the correlations of grain yield are stronger with

some yielding elements and lower with some others. For instance, the grain yield is positive but low correlated with the head diameter according to some authors (Bran, 2022; Clapco et al., 2019) even negative correlated (Mola, 2024), while to some others are positive but significant (Baraiya et al., 2018; Gangavati & Kulkarni, 2021; Machikowa & Saetang, 2008; Nirmala et al., 1999; Patil, 2011; Sanju et al., 2018).

Sunflower crop can be grown over different row spacing which is determining the shape of the nutritional space for a given plant population (Ion et al., 2018). Plant population based on row and plant spacing is a major part of agronomic practices (Beg et al., 2007).

Sunflower yield responses to inter-row distances are variable, there being reports showing that reduced distance between rows could be neutral, beneficial or counterproductive (Calviño et al., 2004).

To achieve highest yield potential of a sunflower hybrid, providing ideal or optimum geometry is through best planting density (Sneha et al., 2022). Grain yield had significant differences by plant density (Modanlo et al., 2021). Even plant spacing effects are highly pronounced in sunflower because there is no possibility of covering gaps between plants by branching or tillering (Sneha et al., 2022), sunflower will compensate to a certain extent for differences in plant populations and density through adjustments in head size (Ibrahim et al., 2023). So, regulating planting density stands out among the critical cultivation factors influencing sunflower yield (Li & Liu, 2025).

Correlation describes the mutual relationship between the variables and helps to improve different characters simultaneously (Singh et al., 2018). Correlation coefficient is very important to define the traits that directly affect the seed yield (Gorgjieva et al., 2015). So, it is of interest from different perspectives (e.g. plant breeding, farming) to identify the correlation of yield with yielding elements according to different growing conditions, both environmentally and technologically. In this respect, the present paper aims to identify the particular correlation of sunflower grain yield with head yielding elements according to row spacing and plant density.

MATERIALS AND METHODS

Research was performed in the years 2019, 2020, and 2021 in four locations in Romania, respectively:

- Negrești, located in eastern part of Romania, in Vaslui County.
- Cogealac, located in southeast part of Romania, in Constanța County.
- Dâlga, located in southern part of Romania, in Călărași County.
- Troian, located in southern part of Romania, in Teleorman County.

Research consisted of field experiments under rainfed conditions organised as subdivided plots with 3 replications. There were studied three experimental factors, respectively:

- Factor A – row spacing, with 3 graduations:
  - a1 = 70 cm;
  - a2 = 60 cm;
  - a3 = 50 cm.
- Factor B – plant density, with 3 graduations:
  - b1 = 50,000 plants/ha;
  - b2 = 60,000 plants/ha;
  - b3 = 70,000 plants/ha.
- Factor C – sunflower hybrid, with 4 graduations:
  - c1 = KWS Acer (early Clearfield hybrid);
  - NK Neoma (mid-early Clearfield hybrid);
  - P64LE25 (mid-early sulfonylurea resistant hybrid);
  - Subaro (mid-late sulfonylurea resistant hybrid).

The crop technology was the same in all locations and experimental years. Thus, the preceding crop was winter wheat.

Fertilisation consisted of applying 40-60 kg/ha of nitrogen and 40-60 kg/ha of phosphorus by spreading 200-300 kg/ha of 20:20:0 complex fertiliser before seedbed preparation, according to soil conditions in each location.

Tillage consisted in ploughing in autumn, one disk harrow passage in March, and seedbed preparation before sowing.

Sowing was performed in the first two decades of April except for Troian location where sowing was performed either at the end of April or at beginning of May, and Dâlga location in 2021, when sowing was performed at beginning of May (Table 1).

Table 1. Sowing data according to location and year

Location	Year		
	2019	2020	2021
Negrești	09 of April	06 of April	19 of April
Cogealac	11 of April	10 of April	16 of April
Dâlga	10 of April	14 of April	03 of May
Troian	03 of May	29 of April	05 of May

The weed control was performed by applying the herbicide Dual Gold 960 EC (S-metolachlor 960 g/l) in a rate of 1.5 l/ha either before seedbed preparation or after sowing, but before emergence. This was completed by the herbicide Select Super (Clethodim 120 g/l) applied in the growth period of sunflower plants in a rate of 0.8-1.3 l/ha, according to the weeds species (annual or perennial monocotyledonous weeds) identified in the experimental field.

The research covered different climatic conditions in the three years and four locations. Regardless of location and year, the registered temperatures were higher than the multiannual average specific for each location (Table 2). In all locations of research, the year 2020 was the warmest, while the year 2019 was the coldest except for Negresti location where the coldest year was 2021. Among the four locations, the highest average temperatures were registered in Cogealac and Dâlga, while the smallest average temperatures were registered in Troian location. In all locations of research, the year 2021 was the rainiest year, while the warmest year 2020 was the driest (Table 2). Among the four locations, the Negrești and Troian locations were the wettest and the Cogealac and Dâlga were the driest. Dâlga location was exposed to extreme rainfall, with a very dry year 2020 (340.9 mm rainfall) and a very wet year 2021 (851.7 mm rainfall).

The research was performed on a soil of chernozem type in all locations except for Negrești location where the soil is of cambic chernozem type.

Table 2. Temperatures and rainfall in the four locations and the three experimental years

Location	2019*	2020	2021	Multiannual average
Temperatures (°C)				
Negrești	12.9	12.2	12.1	9.5
Cogealac	13.3	14.3	13.4	10.7
Dâlga	13.1	14.2	13.8	11.0
Troian	11.1	12.1	11.5	10.4
Rainfall (mm)				
Negrești	539.8	416.1	634.2	420
Cogealac	362.0	340.0	580.0	352
Dâlga	476.5	340.9	851.7	503.6
Troian	535.0	488.0	639.0	550.1

*The climatic data of the experimental years are presented for the period between September of the previous year to August of the current year, when the sunflower vegetation period generally ends or approach the end for the specific growing conditions in Romania.

The grain yield was determined by harvesting the sunflower heads of each experimental variant in the stage of full maturity. The grain yield was calculated in kg/ha and was expressed at 9% moisture content. The yielding elements (head diameter, number of grains per head, weight of grains per head, 1000-grains weight – TGW) were determined for 10 sunflower heads for each experimental variant.

The correlation coefficients were calculated using the Data Analysis Tools of Microsoft Excel.

RESULTS AND DISCUSSIONS

In the performed research, the sunflower grain yield correlated generally positively with the head yielding elements. There are some exceptions, respectively the very weak negative correlations of yield with head diameter at row spacing of 60 cm and density of 50,000 plants/ha (r = -0.076) and with the number of grains per head at row spacing of 50 cm and 60,000 plants/ha (r = -0.057) (Tables 3-5, Figures 1-3).

Table 3. Correlation coefficient (r) of sunflower yield and head yielding elements at row spacing of 70 cm and at different plant density

50,000 plants/ha					
	Yield (kg/ha)	Head diameter (cm)	No of grains per head	Grain weight per head (g)	TGW (g)
Yield (kg/ha)	1				
Head diameter (cm)	0.431423574	1			
No of grains/head	0.175745388	0.117274076	1		
Grain weight/head (g)	0.513454986	0.180229319	0.570566378	1	
TGW (g)	0.389130328	0.189169027	-0.112894462	0.396766194	1
60,000 plants/ha					
	Yield (kg/ha)	Head diameter (cm)	No of grains per head	Grain weight per head (g)	TGW (g)
Yield (kg/ha)	1				
Head diameter (cm)	0.356366839	1			
No of grains/head	0.288845440	0.157853013	1		
Grain weight/head (g)	0.658864581	0.075407149	0.531337823	1	
TGW (g)	0.588680715	0.371253973	0.055254295	0.408685806	1
70,000 plants/ha					
	Yield (kg/ha)	Head diameter (cm)	No of grains per head	Grain weight per head (g)	TGW (g)
Yield (kg/ha)	1				
Head diameter (cm)	0.517011964	1			
No of grains/head	0.400992154	0.441074204	1		
Grain weight/head (g)	0.775304994	0.214372790	0.358322189	1	
TGW (g)	0.608554930	0.608703411	0.138815241	0.350848041	1

Table 4. Correlation coefficient (r) of sunflower yield and head yielding elements at row spacing of 60 cm and at different plant density

	Yield (kg/ha)	Head diameter (cm)	No of grains per head	Grain weight per head (g)	TGW (g)
50,000 plants/ha					
Yield (kg/ha)	1				
Head diameter (cm)	-0.076880	1			
No of grains/head	0.054261	-0.042610	1		
Grain weight/head (g)	0.310268	-0.176700	0.272373	1	
TGW (g)	0.269129	0.149001	-0.136340	0.361068	1
60,000 plants/ha					
Yield (kg/ha)	1				
Head diameter (cm)	0.205915	1			
No of grains/head	0.077994	-0.103230	1		
Grain weight/head (g)	0.579093	-0.012580	0.606142	1	
TGW (g)	0.697858	0.407827	-0.324450	0.261131	1
70,000 plants/ha					
Yield (kg/ha)	1				
Head diameter (cm)	0.140594	1			
No of grains/head	0.200940	0.038788	1		
Grain weight/head (g)	0.676111	0.053928	0.545803	1	
TGW (g)	0.621152	0.296089	-0.073380	0.302772	1

Table 5. Correlation coefficient (r) of sunflower yield and head yielding elements at row spacing of 50 cm and at different plant density

	Yield (kg/ha)	Head diameter (cm)	No of grains per head	Grain weight per head (g)	TGW (g)
50,000 plants/ha					
Yield (kg/ha)	1				
Head diameter (cm)	0.137141	1			
No of grains/head	0.153323	0.227200	1		
Grain weight/head (g)	0.072822	-0.173630	0.32074	1	
TGW (g)	0.343670	0.214444	-0.20684	0.286973	1
60,000 plants/ha					
Yield (kg/ha)	1				
Head diameter (cm)	0.155052	1			
No of grains/head	-0.057520	0.374081	1		
Grain weight/head (g)	0.359730	-0.144600	0.03697	1	
TGW (g)	0.610232	0.306703	-0.32068	0.179304	1
70,000 plants/ha					
Yield (kg/ha)	1				
Head diameter (cm)	0.352839	1			
No of grains/head	0.138444	0.453346	1		
Grain weight/head (g)	0.486980	0.091030	0.021599	1	
TGW (g)	0.548402	0.341524	-0.223650	0.286201	1

Generally, the highest correlation coefficient of grain yield was registered with grain weight per head and TGW regardless of row spacing and plant density, the correlations being moderate and strong. The exception for the grain weight per head was the weak correlations registered at row spacing of 60 cm and density of 50,000 plants/ha and at row spacing of 50 cm and density of 60,000 plants/ha, as well as the very weak correlation registered at 50 cm and density of 50,000 plants/ha. The exception for TGW was the weak correlations registered at row spacing of 60 cm and density of 50,000 plants/ha and at row spacing of 70 cm and density of 50,000 plants/ha (Tables 3-5, Figures 1-3).

These findings are according to those of Marinkovic (1992) who found that the highest direct positive effect on grain yield per plant was exhibited by the weight of 1,000 grains. Also, Öztürk and Ada (2009) found a positive statistically significant relationship between seed yield and 1000-grain weight, while Kang and Ahman (2014) found the strongest correlation of seed yield with 1000-grain weight (r = 0.762) at genotypic level. The results obtained by Mogali and Virupakshappa (1994) revealed that seed yield had highest correlation with number of filled seeds per plant, followed by seed filling percentage and head diameter.

At row spacing of 70 cm, the highest correlation coefficient of grain yield was registered with grain weight per head regardless of plant density.

At row spacing of 60 cm, the highest correlation coefficient of grain yield was registered with grain weight per head at densities of 50,000 and 70,000 plants/ha, but at plant density of 60,000 plants/ha the highest correlation coefficient of grain yield was registered with TGW.

At row spacing of 50 cm, the highest correlation coefficient of grain yield was registered with TGW regardless of plant density.

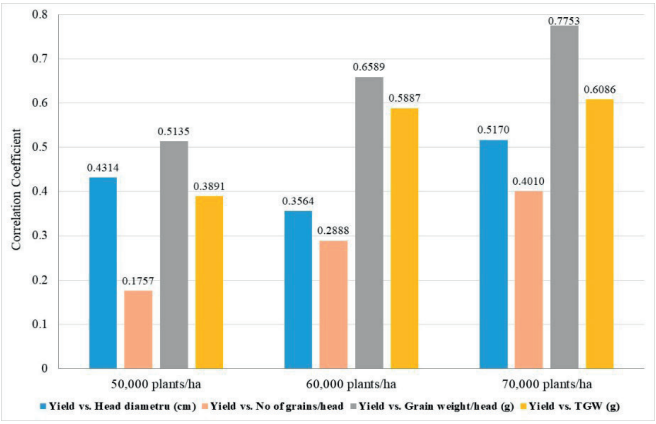


Figure 1. Correlation coefficient (r) of sunflower yield with head yielding elements at row spacing of 70 cm and at different plant density

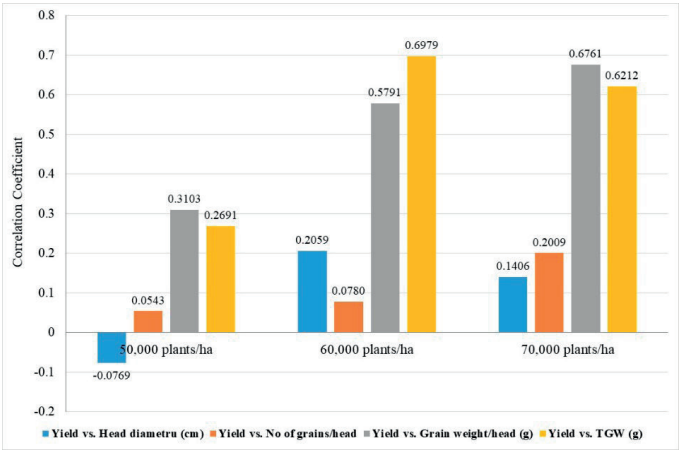


Figure 2. Correlation coefficient (r) of sunflower yield with head yielding elements at row spacing of 60 cm and at different plant density

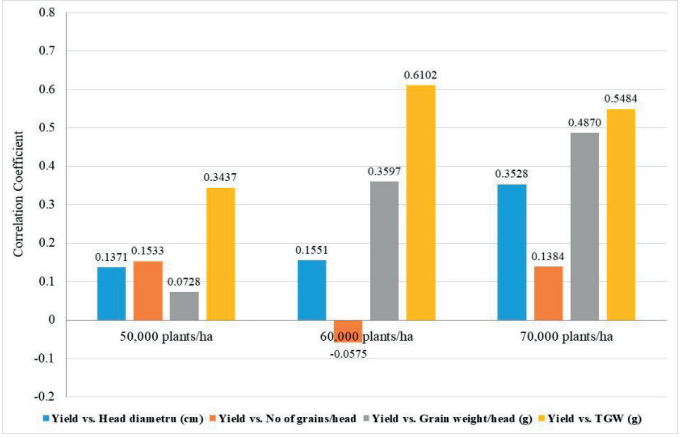


Figure 3. Correlation coefficient (r) of sunflower yield with head yielding elements at row spacing of 50 cm and at different plant density

The smallest coefficient of correlation of grain yield was registered with the number of grains per head at row spacing of 70 cm and 50 cm, and at row spacing of 60 cm and density of 60,000 plants/ha. Also a small coefficient of variation of grain yield was registered with the head diameter, especially in the case of row spacing of 60 cm and densities of 50,000 and 70,000 plants/ha (Tables 3-5, Figures 1-3).

The findings regarding the weak association of the grain yield with the head diameter, even a negative one in the case of the row spacing of 60 cm and density of 50,000 plants/ha are in accordance with those of Hladni et al. (2004) who found that the head diameter has a negative direct effect on seed yield per plant.

Generally, the coefficient of correlation of sunflower grain yield with the head yielding elements decreases with row spacing decrease and increases with plant density increase. In this respect and regarding the TGW, these findings are according to those of Dušanic et al. (2004) who found positive correlations between 1000-seed weight and seed yield, but a highly significant one was found only in the maximum density.

CONCLUSIONS

In the performed research, generally, the coefficient of correlation of sunflower grain yield with the head yielding elements decreases with row spacing decrease and increases with plant density increase.

The highest correlation coefficient of grain yield was registered with grain weight per head and TGW regardless of row spacing and plant density, the correlations being moderate and strong. But regardless of plant density, as the row spacing decreases, it decreases the value of the correlation coefficient of grain yield with grain weight per head and increases the value of the correlation coefficient of grain yield with TGW.

The smallest coefficient of correlation of grain yield was registered with the number of grains per head, and secondly with the head diameter, regardless row spacing and plant density.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

INFLUENCE OF ENVIRONMENTAL CONDITIONS ON RHEOLOGICAL PARAMETERS IN SEVERAL ROMANIAN WINTER WHEAT VARIETIES RELEASED BY NARDI FUNDULEA

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Abstract

*Bread-making quality is a major objective in wheat breeding at NARDI Fundulea. For advancing towards this aim, we use the Reomixer, a device that provide parameters that describe rheological behavior of the dough. We analyzed Reomixer parameters of nine cultivars grown in twenty environments (locations*years) and found the largest variation for parameters "breakdown" and "enwidth" (describing the breakdown of the dough phase) and "peaktime" (describing the dough development phase). Smallest variation was found for "initslope" (describing the water absorption phase) and for grain protein concentration (GPC), while dough strength ("peakheight") and estimated bread volume had intermediate variability. Environments were the main source of variation for GPC, "peakheight" and bread volume, while cultivars had larger influence for "breakdown" and "peaktime". None of the weather parameters analyzed explained more than 10% of the variation in rheological parameters, but in combination produced the observed large variation. Cultivars Pitar, FDL Columna and Voinic had the best average values for GPC, dough strength and bread volume. Our results are useful for wheat breeding programs, opening prospects of breeding for stability of bread-making quality.*

Key words*: wheat, environmental conditions, rheological parameters*.

INTRODUCTION

Wheat is a major crop contributing to the nutrient supply both worldwide and in Romania. A large part of wheat production is used for making bread, and this makes breadmaking quality an important breeding objective. A flour suitable for bread making was defined as having high water absorption, medium to medium-long mixing requirement, satisfactory mixing tolerance, good loaf volume potential and a loaf with good internal crumb grain appearance and color (Finney, 1965). As such, bread-making quality is a complex character depending mostly, but not only, on grain protein concentration (GPC) and subunit composition of the proteins (Payne et al., 1984). A strong positive correlation exists between grain protein concentration and the volume of baked loaves (Finney, 1985), as well as between bread-

making quality and particular subunits of glutenin and gliadins (Payne et al., 1984; Sozinov and Poperelya, 1982; Johansson et al., 1999).

Many studies have examined the effects of genotype and environmental conditions during grain development (Peterson et al., 1997; Graybosch et al., 1995; Mikhaylenko et al., 2000; Panozzo and Eagles, 2000; Preston et al., 2001; Yong et al., 2004). The results of these studies have shown that environment, genotype, and genotype by environment (G × E) interactions are all significant factors contributing to variation in quality. However, most of these studies have indicated that environment is the main contributing factor to variation in quality while G × E interaction contributes a relatively small portion to that variation (Finlay et al., 2007).

Among the methods used to estimate bread-making quality during the wheat breeding process, the Reomixer (Bohlin, 2007) is a convenient way to characterize the mixing properties of the dough (Sedláček and Horčička, 2014). Neacşu et al. (2009) found that most information contained in this large number of mixing parameters can be condensed by five parameters, which describe the basic rheological aspects of dough development and are most appropriate for use in breeding: initial slope - "initslope" (IS) describing the water absorption phase; development time - "peaktime" (PT), describing the mixing requirements of the dough; peak height "peakheight" (PH) describing the dough strength or elasticity; dough breakdown - "breakdown" (BD), describing the dough stability or tolerance to over-mixing; and final width - "endwidth" (EW), describing mainly the dough extensibility. The Reomixer software also estimates the bread volume (BV)

This paper reports an analysis of the effects of environments and genotypes on the rheological parameters measured with the Reomixer.

MATERIALS AND METHODS

We analyzed the above mentioned main Reomixer parameters for 180 samples (9 cultivars released by NARDI Fundulea, tested in twenty yield trials conducted all over Romania). Grain Protein concentration (GPC) was analyzed spectrophotometrically using the FOSS Infratech 1241.

The testing sites covered a large diversity of soils, from chernozems to luvisoils, and of weather conditions, which during the period 2021-2024 reflected present climate changes and were diverse, as illustrated by rainfall during the vegetation period, which varied from 211.2 to 613.8 mm. Crop management of these trials was the one recommended for each environment, including the recommended Nitrogen fertilization (which varied from 82 to 143 kg N/ha).

We analyzed data using ANOVA, regression, and correlations. Regressions on average values of the trial were used to analyze cultivar response to environment (Finlay and Wilkinson, 1963). We used weather data on rainfall and temperatures to study relationships with Reomixer parameters.

RESULTS AND DISCUSSIONS

All analyzed parameters showed exceptionally large variations among the 180 samples (Table 1). The largest variation was found for Breakdown and Peaktime, and the smallest for Grain protein concentration and Initslope.

Table 1. Variation of Reomixer parameters

	BV	GPC	IS	PT	PH	BD	EW
s%	16.96	11.4	11.64	44.32	17.04	98.83	32.48
Max.	1247.5	16.9	9.7	10.0	7.0	5.5	2.95
Min.	512	9.0	5.1	1.3	2.9	0	0.45
Amplitude	735.5	7.9	4.6	8.7	4.1	5.5	2.5
Amplitude, %	85.98	61.4	62.5	181.8	85.1	509.5	172.4

Environments were the main source variation for Bread volume, GPC and Peakheight, while cultivars contributed most to the variation of Peaktime and Breakdown (Table 2). The interaction Cultivar*Environment had a smaller contribution, being the main source variation only for Endwidth.

Table 2. Sources of variation contribution to the variation of Reomixer parameters (%),

	BV	GPC	IS	PT	PH	BD	EW
Environments	**59.6**	**76.6**	**41.7**	29.7	**57.5**	18.1	26.3
Cultivars	12.8	4.2	21	**43.4**	15.2	**43.5**	34
C*Env	27.6	19.2	37.3	26.9	27.3	38.4	**39.7**

Figures 1-4 illustrate the different contribution of the three sources of variation to the total variation of different parameters.

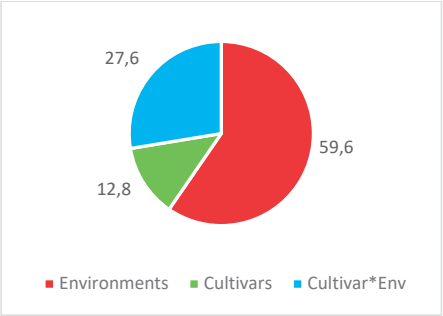


Figure 1. Sources contribution to the variation of BV

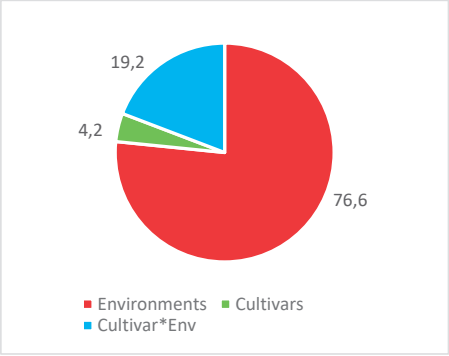


Figure 2. Sources contribution to the variation of GPC

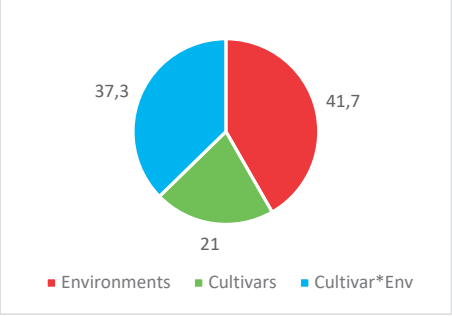


Figure 3. Sources contribution to the variation of IS

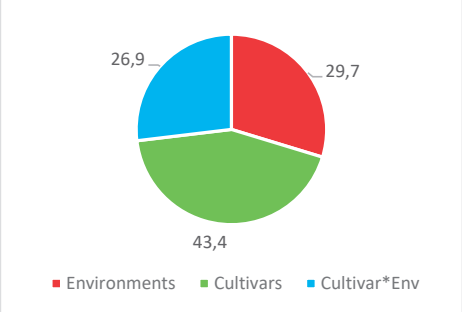


Figure 4. Sources contribution to the variation of Peaktime

For the analyzed samples, grain yield was not significantly correlated with rheological parameters (Table 3), except for the phenotypic negative correlation between yield and both IS and BD.

Table 3. Correlations between yield and quality parameters

	Yield	*GPC*	*IS*	*PT*	*PH*	*BD*	*EW*
GPC	*0.02*	1					
	0.25	1					
IS	*-0.20*	-0.23	1				
	-0.57	0.40	1				
PT	*0.18*	0.64	**-0.80**	1			
	0.40	0.24	**-0.50**	1			
PH	*-0.47*	0.60	0.45	-0.11	1		
	0.08	**0.85**	**0.65**	0.04	1		
BD	*-0.13*	**-0.74**	**0.76**	**-0.95**	-0.10	1	
	-0.54	-0.11	**0.61**	**-0.54**	0.28	1	
EW	*0.13*	**0.74**	**-0.80**	**0.97**	0.07	**-1.00**	1
	0.35	0.37	-0.20	**0.66**	0.23	**-0.70**	1
BV	*-0.37*	**0.82**	0.06	0.26	**0.91**	-0.5	0.44
	0.23	**0.92**	**0.49**	0.17	**0.97**	0.11	0.32

Italic = genetic correlations;
Normal = phenotypic correlations;
Bold = significant at P< 5%.

The nonsignificant correlation between grain yield and GPC was unexpected and might be due to the limited yield variation in the analyzed trials but can also suggest that combining yield potential with quality can be feasible to some extent.

Figure 5 illustrates the large cultivar differences in the relationship between yield and protein concentration.

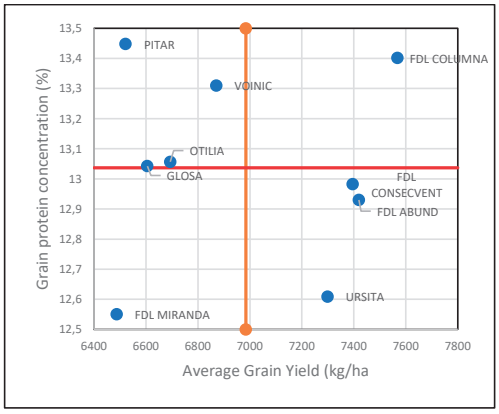


Figure 5. Relationship between grain yield and protein concentration in nine wheat cultivars

If the cultivar Pitar which had the highest protein content was among the lowest yielding cultivars, FDL Columna, the second highest in grain

protein concentration had the highest average yield. Cultivars FDL Consecvent and FDL Abund, which were placed second and third in yield had average grain protein percentage, while FDL Miranda and Ursita with the lowest grain protein concentrations had contrasting yields.

GPC was strongly correlated with bread volume, both genetically and phenotypically, with protein concentration explaining more than 65% of the bread volume variation. Higher protein content was also associated with better dough strength (PH).

Fast water absorption (high IS) was strongly associated with reduced mixing requirements (small PT) and with low dough stability (high BD), both between cultivars and between environments, High mixing requirements (PT) were associated with dough stability (low BD) and with better dough extensibility (high EW).

High dough stability showed perfect genetic association with low dough extensibility (correlation of r =-1 between BD and EW), which suggests common genetic control.

Cultivars were significantly different in all analyzed quality parameters (Table 4).

Cultivar Pitar ranked first for bread volume, grain protein concentration and dough strength (peak height), while Glosa ranked first for water absorption, Otilia for peak time and FDL Abund for dough stability (low breakdown value).

Table 4. Average reomixer parameters in nine wheat cultivars

	BV	GPC	IS	PT	PH	BD	EW
Pitar	**933**	**13.45**	7.58	6.12	**5.33**	0.64	1.68
Voinic	907	13.31	6.95	5.71	4.99	0.7	1.63
Glosa	906	13.04	**7.85**	3.49	5.25	1.56	1.25
FDL Columna	898	13.40	7.38	5.48	5.01	0.58	1.64
FDL Abund	842	12.93	6.86	6.37	4.60	**0.50**	1.73
Otilia	833	13.06	6.68	**7.07**	4.47	0.25	**1.80**
Miranda	829	12.55	7.59	2.97	4.8	1.94	1.11
FDL Consecvent	803	12.98	7.23	5.52	4.46	0.83	1.51
Ursita	771	12.61	7.75	3.31	4.48	2.43	1
Average	858	13.04	7.32	5.12	4.82	1.05	1.48

The analyzed cultivars responded differently to environmental conditions. To characterize the cultivar specific response to the environment we calculated the amplitude of variation and analyzed the regression of traits for each cultivar on the average value of all cultivars in the trial, as recommended by Finlay and Wilkinson (1963). Cultivars combining high average values with low variation amplitudes and regression slopes, as well as positive intercepts would be desirable having better stability.

This type of analysis is presented in table 5 for bread volume.

Table 5. Response of BV to environmental conditions in nine wheat cultivars

	Average BV	Amplitude	b	a
Pitar	933	495	1.152	-56.2
Voinic	907	426	0.922	115.5
Glosa	906	479	0.889	143.6
FDL Columna	898	624	1.117	-60.4
FDL Abund	842	539	1.12	-119.2
Otilia	833	617	1.304	-285.8
FDL Miranda	829	536	0.94	22.7
FDL Consecvent	803	552	0.985	-41.7
Ursita	771	316	0.571	280.8

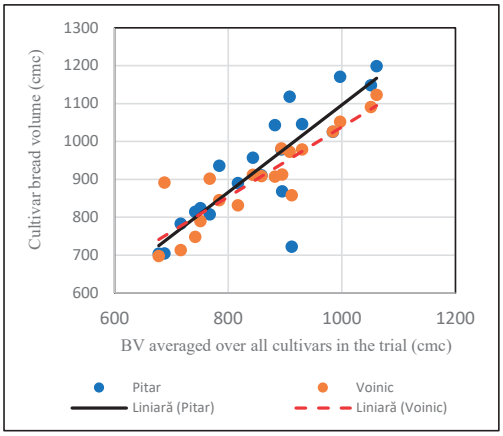


Figure 6. Bread volume variation in cultivars Pitar and Voinic

Figure 6 illustrates the difference between cultivars Pitar and Voinic, both having high values of bread volume, the last one responding less to environmental variation. Cultivars Otilia and FDL Miranda also showed different responses to environment, while having similar average bread volume (Figure 7).

The same type of analysis of cultivar response to environment is presented in Table 6 for grain protein concentration.

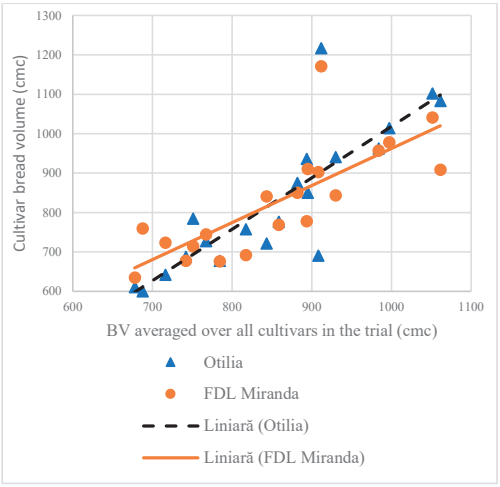


Figure 7. Bread volume variation in cultivars Otilia and FDL Miranda

The same type of analysis of cultivar response to environment is presented in Table 6 for grain protein concentration.

Table 6. Response of GPC to environmental conditions in nine wheat cultivars

	Average GPC	Amplitude	b	a
Voinic	13.3	4.4	0.739	3.70
Glosa	13.0	3.7	0.856	1.91
FDL Miranda	12.6	4.3	0.901	0.84
Ursita	12.6	4.5	0.911	0.76
Otilia	13.1	4.5	0.918	1.12
FDL Columna	13.4	6.1	1.055	-0.31
Pitar	13.4	5.8	1.066	-0.41
FDL Abund	12.9	6.7	1.097	-1.33
FDL Consecvent	123	7.1	1.197	-2.59

The amplitude of variation between the maximum and minimum values recorded for each cultivar varied from 3.7% in cultivar Glosa to 7.1% in FDL Consecvent. The regression slope varied from 0.739 to 1.197 and the regression intercept varied from +3.70 to -2.59. Figure 8 illustrates the response of GPC to environmental conditions in two contrasting cultivars, Glosa and FDL Consecvent.

We observed contrasting cultivar responses to environment for dough stability (Table 7).

Cultivar FDL Abund, with good average dough stability, showed almost constant Breakdown value in all twenty trials, while Ursita, with the lowest average dough stability, showed exceptionally large BD variation (Figure 9).

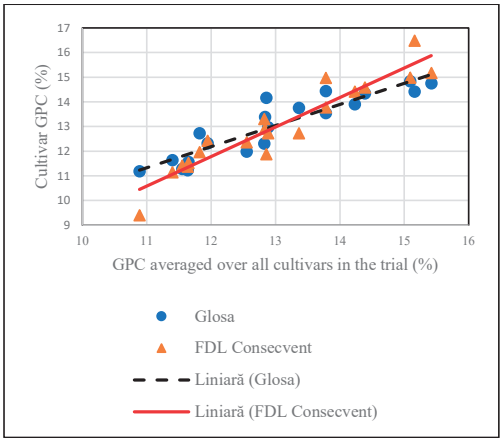


Figure 8. Variation of GPC in cultivars Glosa and FDL Consecvent

Table 7. BD response to environmental conditions in nine wheat cultivars

	Average BD	Amplitude	b	a
Otilia	0.25	0.98	0.203	0.04
FDL Abund	0.36	0.99	0.217	0.13
FDL Columna	0.58	1.46	0.729	-0.17
Pitar	0.64	1.53	0.644	-0.03
Voinic	0.69	3.6	1.192	-0.53
FDL Consecvent	0.83	3.37	0.825	-0.02
Glosa	1.56	3.46	1.507	0.01
FDL Miranda	1.93	3.28	1.045	0.86
Ursita	2.42	5.43	2.636	0.29

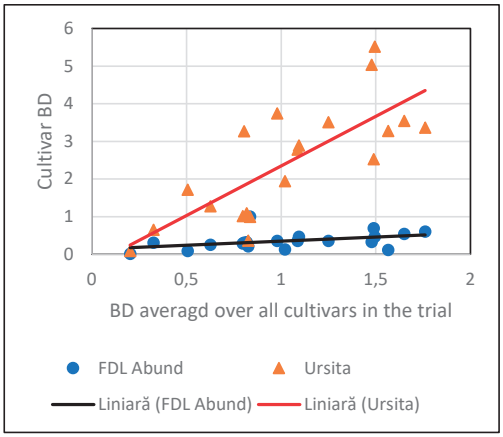


Figure 9. Variation of BD in cultivars Ursita and FDL Abund

We could not associate the variation of Reomixer parameters with specific weather data, as none of the simple, brief period analyzed weather parameters explained more than 10% of the variation in rheological results.

CONCLUSIONS

We found exceptionally large variation of all Reomixer parameters in the one hundred eighty samples analyzed, with the largest variation found for Breakdown and Peaktime, and the smallest for Grain protein concentration and Initslope. Environments were the main source of variation for GPC, "peakheight" and bread volume, while cultivars had larger influence for "breakdown" and "peaktime". The interaction between cultivars and environments was the main source of variation for dough extensibility (EW).

Grain yield showed no genetic correlation with the Reomixer parameters. High grain protein concentrations were found in low yielding cultivars, but also in cultivar FDL Columna, which produced the highest average yield. Phenotypic correlations were significant only with IS and BD.

GPC was strongly correlated genetically and phenotypically with bread volume and with dough strength (PH), while fast water absorption (high IS) was strongly associated with reduced mixing requirements (small PT) and with low dough stability. Mixing requirements (PT) was negatively associated with dough stability and positively with dough extensibility.

The analyzed cultivars responded differently to environmental conditions as shown by contrasting amplitudes of variation, slopes, and intercepts of Finlay-Wilkinson regressions. This finding opens prospects for breeding to improve bread-making quality stability in diverse environmental conditions.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

EVALUATION OF SOIL AND FOLIAR HERBICIDAL APPLICATIONS AT CAMELINA (*Camelina sativa* (L.) Crantz

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Abstract

Camelina is one of the underestimated oil crops with high potential for low input production. Main constrain during the cultivation is the weed infestation. A field trial for evaluation of the efficacy and the selectivity of several herbicides applied on three genotypes (variety Czenstochowska, local landrace BGR 436, and introduced accession CAM 265) was performed at the Agricultural University of Plovdiv, Bulgaria in 2022 and 2023. The herbicides Stomp Aqua (455 g/l pendimethalin) – 4.00 L ha^{-1} and Butisan 400 SC (400 g/l metazachlor) – 2.50 L ha^{-1} were applied after sowing before germination. The studied foliar herbicides were Lontrel 300 SL (300 g/l clopiralid) – 0.40 L ha^{-1} and Galera Super (240 g/l clopyralid + 80 g/l picloram + 40 g/l aminopyralid) – 0.20 L ha^{-1}. The products were sprayed at the growth stage when a single true leaf on 5th to 8th node was developed on the crop. The Lontrel 300 SL and Galera Super treatments showed higher weed control compared to Stomp Aqua and Butisan 400 SC, but had higher phytotoxixity as well that resulted in growth retardation and yields decrease.

Key words*: Camelina, herbicides, weeds, plant development, yields.*

INTRODUCTION

Camelina sativa origin is from south-eastern Europe and south-western Asia and is a member of the Brassicaceae family (Singh et al., 2015). *Camelina sativa* is also known as false flax or gold-of-pleasure (Luo et al., 2019). Camelina is one of the underestimated oil crops around the globe as well as in Bulgaria and neighbouring countries, but in the last decade is gaining great interest. The increased industrial search for alternatives to fossil fuels for jet fuel and biodiesel can be answered with this easy-to-grow and low-input crop well-adapted to each environment (Bakhshi et al., 2021; Mondor and Hernández-Álvarez, 2022). One of the greatest problematic issues during camelina growing is the weed infestation (Lenssen et al., 2012). One of the approaches for weed control in camelina is growing the crop as a mixed cropping system with legumes that leads to the suppression of some weeds (Saucke and Ackermann, 2006; Neshev et al., 2023). According to Codina-Pascual et al. (2022), the camelina can suppress the development of corn poppy and its seed production and may be considered a tool for IWM. In nowadays agriculture, weed management is based on herbicidal treatments (Simic et al., 2011; Mitkov et al., 2016, Jursík et al., 2017; Neshev et al., 2020). The weed management with herbicides has to be performed in the optimal crop growth stage (Simic et al., 2011). Therefore, a low number of herbicidal products are registered for application in the camelina fields. That makes weeds a difficult problem during the cultivation of the crop (Sobiech et al., 2020).

The application of the herbicides is not effective enough for full weed control and the crop is tolerant to a limited number of herbicides for broadleaf weed control (Berti et al., 2016; Obour, 2015). On the other hand, most of the herbicidal products applied before germination of the camelina led to injuries on the crop (Jha and Stougaard, 2013; Scheliga and Petersen, 2016).

Herbicide phytotoxicity can be observed in different situations as application of the herbicides in unsuitable weather conditions (waterlogging, drought, low or high temperatures, etc.) and herbicide drift; In case of technological mistakes by increasing the herbicidal rate; Application of herbicide on a sensitive by mistake; Due to insufficient selectivity; etc. (Hanks, 1995; Vischetti et al., 2002; Thistle, 2004; Neshev et al., 2022).

According to Dayan et al. (2015) and Dayan and Zaccaro (2012) if the detoxification of the

herbicide sufficient different functional impairments appears in plants. The herbicidal toxicity is most often chronic, but in some situations, it may be lethal for the crop.

Prashant and Stougaard (2013) evaluated the tolerance of camellia to various herbicides such as quinclorac, S-metolachlor, dimethenamid-P, pendimethalin, and pyroxasulfone. Quinclorac applied before emergence did not cause significant damage to the camelina and had no negative effect on plant density, biomass, flowering, and yield. S-metolachlor caused less than 20% damage to the camelina. Dimethenamid-P applied at 630 g ai ha^{-1} did not affect camelina seeding density, nor biomass, flowering, and yield; however, at a rate of 1,260 g ha^{-1} the damage in sandy soils was up to 60%, flowering was delayed, and plant density and yield were reduced by 50 and 31%, respectively. Although it caused some visual damage to camelina, crop yield was not reduced by pendimethalin.

The results obtained in Russia by Serdyuk et al. (2021) are contradictory. The application of combinations of herbicides such as clopyralid, S-metolachlor, ethametsulfuron-methyl a significant reduction in plant density (by 18-32 plants m^2) and seed yield (by 0.33-0.52 t ha^{-1}) of camelina compared to the control was reported.

The ability to recover the herbicide-damaged plants depends on the degree of the occurred structural-functional impairment. Some studies have shown that chronic herbicide phytotoxicity can be overcome (to some extent or completely) by the application of biostimulants, foliar fertilizers, growth regulators, herbicide antidotes, etc. (Jablonkai, 2013; Balabanova et al., 2016; Colla et al., 2017; Balabanova, 2021; Soltani, 2015; Neshev et al., 2020; Neshev et al., 2021; Neshev et al., 2022).

There is still limited information about the herbicidal treatments' influence on the growth and development of camelina landraces. Therefore, this study aimed to evaluate the efficacy against the weeds of different herbicide products and evaluate the influence of the herbicidal treatments on camelina genotypes.

MATERIALS AND METHODS

The field study was carried out at the experimental field of the Agricultural University - Plovdiv, Bulgaria in 2022 and 2023. The experiment was done by the randomized block method in three repetitions. The size of the experimental plot was 10 m^2.

The plants were sown at a density of 500 germinated seeds/m^2 at the beginning of March, with a plot seeder. In both years of the research, the preceding crop of camelinas was sunflower. On the field of the trial deep ploughing, two times disc harrowing, and two times cultivation before sowing were done. After sowing before germination fertilization with 150 kg ha^{-1} with combined NPK fertilizer (15:15:15) was accomplished. No insecticides and fungicides were applied before or during the vegetation. No irrigation is provided for emergence or during the two growing seasons.

The herbicides Stomp Aqua and Butizan 400 SC were applied after sowing before germination (BBCH 00). The products Lontrel 300 SL and Galera Super were administered in the phenophase rosette (BBCH15-18). The treatments were performed with backpack sprayer SOLO model 417 (Solo, Germany). The size of the working solution was 250 L ha^{-1}.

Variants of the trial were:

1. Untreated control;
2. Stomp Aqua – 4.00 L ha^{-1};
3. Butizan 400 SC – 2.50 L ha^{-1};
4. Lontrel 300 SL – 0.40 L ha^{-1};
5. Galera Super – 0.20 L ha^{-1}.

Used herbicidal products:

- Stomp Aqua (455 g/l pendimethalin). Stomp® Aqua is a herbicide for the control of annual grass and broad-leaved weeds in a wide range of crops such as wheat, barley, rye, transplanted brassicas, carrots, onions, parsnips, combining peas, potatoes, sunflowers, triticale, etc. (https://www.agricentre.basf.co.uk/en/Products/Product-Search/Herbicides/Stomp-Aqua.html).
- Butizan 400 SC (400 g/l metazachlor). Butisan 400 SC is a selective herbicide for soil and early vegetation application against annual broadleaf and grass weeds. It is registered to be applied in cabbage, cabbage seedlings (beds), and oilseed rape (https://agroconsult-buinov.com/en/produkt/butisan-400-sc/).
- Lontrel 300 SL (300 g/l clopyralid) – 0.40 ml ha^{-1}. It is a post-emergence herbicidal product that can be applied in onions, grapevines, wheat, maize, mustard sugar beet, etc. It controls broadleaf weed species only

(https://www.agropataki.ro/en/herbicide/lontrel-300-ec-p19156.html).

- Galera Super (240 g/l clopyralid + 80 g/l picloram + 40 g/l aminopyralid) – 0.20 ml ha^{-1}. It is a foliar herbicide that controls annual and perennial broadleaf weeds in oilseed rape (https://www.nexles.com/eu/dow-agro-sciences-herbicide-galera-super-1-liter.html).

Herbicidal efficacy was assessed on the 10-score visual scale of EWRS (European Weed Research Society) on the 14th, 28th, and 42nd day after treatment (over 14 days). The efficacy is averaged for the three camelina genotypes since the herbicidal treatments are equal.

Herbicidal selectivity was assessed on the 9-score visual scale of EWRS on the 7th, 14th, 21st, and 28th day after crop post-emergence for the soil treatments (at 7 days) and on the 7th, 14th, 21st, and 28th day after treatment of the variants with foliar herbicides (in 7 days) as described below:

1. No effect (healthy plants);
2. Very slight effects; some stunting and yellowing just visible;
3. Slight effects; stunting and yellowing, effects reversible;
4. Substantial chlorosis and or stunting, most effects probably reversible;
5. Strong chlorosis/stunting; thinning of stand;
6. Increasing severity of damage;
7. Increasing severity of damage;
8. Increasing severity of damage;
9. Total loss of plants and yield.

The natural weed species on the field were Fat-hen (*Chenopodium album* L.) – 21 specimens m^2 in 2022 and 17 specimens m^2 in 2023; Cleavers (*Galium aparine* L.) – 7 specimens m^2 in 2022 and 11 specimens m^2 in 2023; Forking larkspur (*Consolida regalis* Gray.) – 12 specimens m^2 in 2022 and 9 specimens m^2 in 2023; Spiny cocklebur (*Xanthium spinosum* L.) – 8 specimens m^2 in 2022 and 10 specimens m^2 in 2023; Green foxtail (*Setaria viridis* (L.) P. Beauv.) – 14 specimens m^2 in 2022 and 17 specimens m^2 in 2023; Annual ryegrass (*Lolium rigidum* Gaud.) – 16 specimens m^2 in 2022 and 19 specimens m^2 in 2023.

There is no registered camelina variety in Bulgaria, therefore three genotypes *of Camelina sativa* (L.) Crantz with different origins were chosen as test plants – landrace from Poland ("Czenstochowska"), Italy ("CAM 265"), and Bulgaria - local population ("BGR 436"). The "BGR 436" plants are part of a large collection collected through international scientific exchange with gene banks and scientists, within the framework of a project under the National Science Program "Healthy Biofoods for a Strong Economy and Quality of Life". The selection of the specific genotypes is based on different origins, phenological development, and morphological characteristics.

Several observations, biometrical, vegetative, and productive evaluations and measurements were performed:

- Symptoms of herbicidal damage;
- Plant height (PH) at the end of the vegetation (cm). The height of thirty plants per plot was measured (ninety plants total per treatment);
- Number of primary brunches $plant^{-1}$ (NPB $plant^{-1}$) at the end of the vegetation. The number of primary branches of thirty plants per plot was measured (ninety plants total per treatment);
- Number of siliques $plant^{-1}$ (NS $plant^{-1}$) at the end of the vegetation. The number of the siliques of thirty plants per plot was measured (ninety plants total per treatment);
- Seed yield $plant^{-1}$ (SY), g. After manual harvesting, the seeds of thirty separate plants per plot were weighted individually on an electronic scale. The obtained results were summed and divided to thirty to recalculate the average seed yield $plant^{-1}$. Ninety plants total per treatment were analysed.

The analyses were performed separately for each of the three camelina landraces grown in the research.

The agrometeorological data during the experiments was taken from the automatic meteorological station "Meteobot® Pro". The presented data is for the average monthly minimum and maximum air temperatures (°C) as well as precipitation (mm) for the vegetation periods of camalinas (from March to June) during the two trial years.

The statistical analysis of the collected data for the plant height, number of primary branches $plant^{-1}$, number of siliques $plant^{-1}$ at the end of the vegetation as well as the seed yield $plant^{-1}$ was performed by Duncan's Multiple Range test (One-Way ANOVA) by the software program SPSS 26. As the efficacy and the selectivity were evaluated by visual rating no statistical analyses were done.

RESULTS AND DISCUSSIONS

Figure 1 shows the average temperatures, and Figure 2 presents the amount of precipitation during the vegetation period of the camelina genotypes. Temperatures were optimal for the crop development and no negative influence of temperatures on the growth and development of the studied camelina genotypes was found. It is seen that the amount of precipitation during the different months is a prerequisite for relatively good moisture storage and normal vegetation. Camellia was sown on dry soil in March. In both years there was a prolonged period of drought before sowing, but there was heavy rainfall at the end of March. Regarding the moisture during the growing season, in both experimental years, no period in which the camelina genotypes experienced a water deficit was established.

Based on the analysis of the meteorological data, we can point out the two experimental years as relatively favourable for the growth, development, and realization of the productive abilities of the grown camelina genotypes.

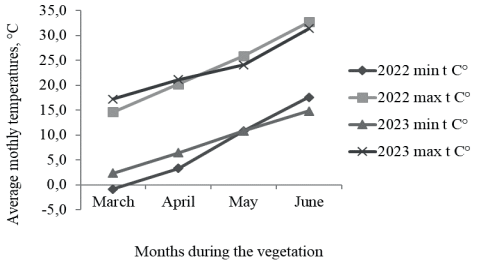


Figure 1. Average monthly minimum and maximum air temperatures, °C

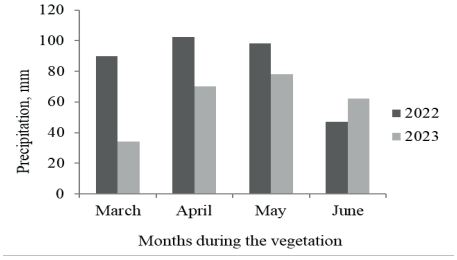


Figure 2. Average monthly precipitation, mm

All pesticides as well as herbicides require attention to meteorological factors such as temperatures and precipitation (World Meteorological Organization, 2010). There were no temperature values reported, which can contribute to a wrong assessment of the herbicidal substances' phytotoxic performances to the crop and the valuation of their efficacy against the weeds. If rain falls at the application time or immediately after that it may lead to washing away any unabsorbed amount of the herbicides applied (Tonev et al., 2011). The amount of precipitation during treatments in both years of the study did not affect the phytotoxic expressions and their overcoming by the camelina genotypes. The efficacy against the weeds was not influenced by the precipitation as well.

The efficacy of the soil-applied herbicide products Stomp Aqua and Butizan 400 SK against all weeds was lower on the first reporting date and increased by the third evaluation date. This is most likely due to several major factors influencing the action of soil-applicable herbicides such as the extent to which the herbicide is adsorbed to soil particles, the rate of absorption of the herbicide solution by plant roots, and the rate at which the herbicide decomposes, volatilizes or leaches from the soil (Osgerby, 1973). In contrast to the soil-applied herbicides, the efficacy of vegetatively applied Lontrel 300 SL and Galera Super against all weeds was lower on the first reporting date and increased by the third reporting date. This corresponds with results reported by different authors on other crop plants (Mitkov et al., 2017; Mitkov et al., 2018; Yanev et al., 2021).

Table 1 shows the efficacy of the evaluated herbicides against the weeds developing together with the camelina landraces.

For the weed Fat-hen (*Ch. album*) the soil-applied herbicides showed 88% efficacy on the 14th day after treatments. On the next reporting date, the efficacy decreased to 68-78% for variant 2 (Stomp Aqua – 4.00 L ha^{-1}) and variant 3 (Butizan 400 SC – 2.50 4.00 L ha^{-1}) respectively. On the 42nd day, the efficacy was lower - 48 - 55%.

In the case of the foliar herbicides, a higher initial efficacy (on the 14th day) was reported for variant 5 (Galera Super – 0.20 4.00 L ha^{-1}) – 65% on average for the two experimental years. The efficacy of Lontrel 300 SL – 0.40 4.00 L ha^{-1} (variant 4) was very low – 25%. At the next reporting date, the efficacy of Galera Super and

Lontrel increased to 45 and 78%, respectively. On the 42nd day, the efficacy of Lontrel 300 SL was 55%, indicating that the herbicide could not control the Fat-hen to a significant economic effect. The efficiency of Galera Super reached 88%, which effectively controlled the weed.

Table 1. Efficacy of the evaluated herbicides against the weeds, %

Treatments/Weeds/Year	2022	2023	Average for the period
Ch. album	(%)	(%)	(%)
1. Untreated control	-	-	-
2. Stomp Aqua – 4.00 L ha^{-1}	85.00	75.00	50.00
3. Butizan 400 SC – 2.50 L ha^{-1}	90.00	70.00	50.00
4. Lontrel 300 SL – 0.40 L ha^{-1}	20.00	40.00	50.00
5. Galera Super – 0.20 L ha^{-1}	60.00	75.00	85.00
G. aparine	(%)	(%)	(%)
1. Untreated control;	-	-	-
2. Stomp Aqua – 4.00 L ha^{-1}	80.00	65.00	50.00
3. Butizan 400 SC – 2.50 L ha^{-1}	85.00	70.00	50.00
4. Lontrel 300 SL – 0.40 L ha^{-1}	60.00	70.00	75.00
5. Galera Super – 0.20 L ha^{-1}	75.00	85.00	90.00
C. regalis	(%)	(%)	(%)
1. Untreated control	-	-	-
2. Stomp Aqua – 4.00 L ha^{-1}	70.00	50.00	30.00
3. Butizan 400 SC – 2.50 L ha^{-1}	80.00	60.00	40.00
4. Lontrel 300 SL – 0.40 L ha^{-1}	40.00	50.00	60.00
5. Galera Super – 0.20 L ha^{-1}	50.00	70.00	90.00
Xa. spinosum	(%)	(%)	(%)
1. Untreated control	-	-	-
2. Stomp Aqua – 4.00 L ha^{-1}	50.00	30.00	25.00
3. Butizan 400 SC – 2.50 L ha^{-1}	40.00	25.00	20.00
4. Lontrel 300 SL – 0.40 L ha^{-1}	45.00	70.00	90.00
5. Galera Super – 0.20 L ha^{-1}	40.00	50.00	60.00
S. viridis	(%)	(%)	(%)
1. Untreated control	-	-	-
2. Stomp Aqua – 4.00 L ha^{-1}	90.00	80.00	50.00
3. Butizan 400 SC – 2.50 L ha^{-1}	80.00	60.00	40.00
4. Lontrel 300 SL – 0.40 L ha^{-1}	0.00	0.00	0.00
5. Galera Super – 0.20 L ha^{-1}	0.00	0.00	0.00
L. rigidum	(%)	(%)	(%)
1. Untreated control	-	-	-
2. Stomp Aqua – 4.00 L ha^{-1}	65.00	45.00	25.00
3. Butizan 400 SC – 2.50 L ha^{-1}	65.00	50.00	30.00
4. Lontrel 300 SL – 0.40 L ha^{-1}	0.00	0.00	0.00
5. Galera Super – 0.20 L ha^{-1}	0.00	0.00	0.00

The efficacy of the tested herbicide against the weed Cleavers (*G. aparine*) was similar to those reported for the Fat-hen. The soil-applied herbicides showed average efficacy from 85 to 88% on the 14th day after application. On the next reporting date, the efficacy decreased to 70 and 73% for variant 2 (Stomp Aqua – 4.00 L ha^{-1}) and variant 3 (Butizan 400 SC – 2.50 L ha^{-1}) respectively. On the 42nd day, the efficacy of the herbicides was lower – 45%. On the 14th day, the foliar application of Galera Super – 0.20 L ha^{-1} (variant 5) reached 78% on average for the two years of the study and the efficacy of Lontrel 300 SL – 0.40 L ha^{-1} (variant 4) was 65%.

At the next reporting date, the efficacy of variants 4 and 5 increased to 73 and 88%, respectively. On the 42nd day after treatment, the efficacy of Lontrel was 80%, and that of Galera Super – 93%.

The efficacy of the evaluated herbicidal products against the weed Forking larkspur (*C. regalis*) was similar to that found in the Fat-hen and Cleavers. For the soil herbicides, the average efficacy on the 14th day after treatment was 75 to 85%. On the next reporting date, the efficacy decreased to 55 and 65% for variant 2 (Stomp Aqua – 4.00 L ha^{-1}) and variant 3 (Butizan 400 SC – 2.50 L ha^{-1}) respectively. On the 42nd day, the efficacy of the herbicides decreased to 35%.

Averaged over the two years, the efficacy of the foliar herbicides against the weed Forking larkspur on the 14th day after treatments was 48% for variant 5 (Galera Super – 0.20 L ha^{-1}).

The efficacy of Lontrel 300 SL – 0.40 L ha^{-1} (variant 4) was 45%. At the next reporting date, the efficacy of treatments 4 and 5 increased to 55 and 68%, respectively. On the 42nd day, the efficacy of Lontrel 300 SL was 68%, and that of Galera Super reached 88%. The efficacy against the weed species Spiny cocklebur (*Xa. spinosum*) for the soil-applied herbicides was lower than that reported for the above-mentioned weed species. The average efficacy on the 14th day was 43 to 45% for variant 3 (Butizan 400 SC – 2.50 L ha^{-1}) and variant 2 (Stomp Aqua – 4.00 L ha^{-1}) respectively. On the next reporting date, the efficacy decreased to 30%, and on day 42nd to 20%.

Averaged over the two years, the efficacy of the foliar herbicides on the 14th day after treatment was 43% for treatment 5 (Galera Super – 0.20 L ha^{-1}). The efficacy of Lontrel 300 SL – 0.40 L ha^{-1} (variant 4) was 45%. At the next reporting date, the efficacy of the treatments increased to 73 and 55%, respectively. On the 42nd day after treatment, the efficacy of Lontrel 300 SL was the highest - 95%, and that of Galera Super was significantly lower - 65%. The efficacy against Green foxtail (*S. viridis*) and Annual ryegrass (*L. rigidum*) only for the soil-applied herbicide products was reported, as the foliar herbicides Lontrel 300 SL and Galera Super control only broadleaf weed species.

The average efficacy against Green foxtail on the 14th day after treatment was 85% for treatment 3 (Butizan 400 SC – 2.50 L ha^{-1}) and treatment 2 (Stomp Aqua – 4.00 L ha^{-1}). At the next evaluation date, the efficacy decreased to 65% in variant 2 and 78% in variant 3. At day 42 after treatments, the efficacy decreased to 45% in both variants of the trial.

The efficacy of Stomp Aqua and Butizan 400 SC against the Annual ryegrass (*L. rigidum*) was lower than that reported against the weed Green foxtail. The reported average efficacy on the 14th day for Butizan 400 SC – 2.50 L ha^{-1}) was 68%, and for Stomp Aqua – 4.00 L ha^{-1}) was 60%. At the next assessment date, efficacy decreased to 43% for treatment 2 and 48% for treatment 3. At day 42 the efficacy decreased to 28% for Stomp Aqua and to 30% for Butizan 400 SC.

The following Table 2 present the results of the observed visual phytotoxicity in the three camelina genotypes. Plants of the three landraces responded relatively equally to the herbicide intervention and exhibited almost identical symptoms of herbicidal stress. The phytotoxic symptoms were more severely pronounced on the first reporting date, after which it was overcome by the plants to some extent depending on the herbicide product applied.

Pendimethalin is a soil-applicable herbicidal active substance from the dinitroaniline chemical group. It has to be applied after sowing before germination of the crop (Smith et al., 1995). The phytotoxic symptoms are root growth retardation and stunning of the sensitive plants. Phytotoxicity of pendimethalin is reported on onions (Acar et al., 2022), pumpkins (Neshev et al., 2019), and camelina (Jha and Stougaard, 2013). In our study, herbicidal toxicity of pendimethalin (Stomp Aqua) was recorded only on the first reporting date (7 days after treatments) (Table 2). After that, the plants overcome the herbicidal stress and develop normally.

The other pre-emergence herbicide evaluated in the study was Butizan 400 SC which contains the active substance metazachlor. The herbicidal active ingredient is an inhibitor of the very long chain fatty acids biosynthesis (Boger, 2003). This herbicide belongs to the chemical group of pyrazoles. Metazachlor can be applied to several crops as winter oilseed rape (Dimitrova et al., 2014), cauliflower (Šuk et al., 2018), kohlrabi (Jursík et al., 2019), etc. In a study conducted by Serdyuk et al. (2021), it was concluded that the application of quinmerac + metazachlor showed no phytotoxic the camelina plants. In our trial, there were herbicidal stress symptoms after the application of Butizan 400 SC. Phytotoxic symptoms were found in all three camelina genotypes. The phytotoxicity recorded on the 7th day after germination of the plants was more severely pronounced and was determined as score 4 (Table 2 and Figure 2). The symptoms of phytotoxicity were expressed in chlorosis (yellowing) and distortion of the leaf petiole and leaf blade. Plants appear stunted. On the second reporting date, the phytotoxicity decreased and reached a score of 2, as the plants overcame the herbicide toxicity. On the 21st and 28th days after emergence, the plants completely overcome the visible symptoms of phytotoxicity.

Clopyralid is the active substance from the group pyridine carboxylates and it is known as

synthetic auxin (Busi et al., 2018). Clopiralid (the active substance of the herbicidal product Lontrel 300 SL) can be applied in oilseed rape (Blackshaw, 1989; Mitkov et al., 2018), strawberries (Dobre et al., 2018), winter wheat (Gallo and Pekár, 2001.), onions (Gidea, M., 2014), etc. The camelina landraces were more severely damaged by the application of the foliar herbicides and experienced the stress to a higher degree. It was also reported in a research conducted by Sobiech et al. (2020) where the application of clopyralid damaged the evaluated plants to a greater extent than the other studied herbicides (propaquizafop, quizalofop-p-ethyl, and picloram). For Galera Super, the other herbicidal product studied in our trial, information for its application in camelina was not found. The combination of the three active substances (clopyralid + aminopyralid + picloram) in the commercial formulation showed stronger phytotoxicity to the three camelina genotypes when compared to the other treatments. The herbicide is applied for weed control in oilseed rape (Mitkov et al., 2017).

Table 2. Visual phytotoxicity in the three camelina genotypes, scores by EWRS

Treatments/Years/Days after treatments	2022				2023			
	7 days	14 days	21 days	28 days	7 days	14 days	21 days	28 days
"Czenstochowska"								
1. Untreated control	-	-	-	-	-	-	-	-
2. Stomp Aqua – 4.00 L ha^{-1}	2	1	1	1	2	1	1	1
3. Butizan 400 SC – 2.50 L ha^{-1}	4	3	1	1	4	3	1	1
4. Lontrel 300 SL – 0.40 L ha^{-1}	5	4	2	2	5	4	2	2
5. Galera Super – 0.20 L ha^{-1}	6	4	3	2	6	4	3	2
"CAM 265"								
1. Untreated control	-	-	-	-	-	-	-	-
2. Stomp Aqua – 4.00 L ha^{-1}	2	1	1	1	2	1	1	1
3. Butizan 400 SC – 2.50 L ha^{-1}	4	2	1	1	4	2	1	1
4. Lontrel 300 SL – 0.40 L ha^{-1}	6	5	3	2	6	5	3	2
5. Galera Super – 0.20 L ha^{-1}	6	5	3	3	6	5	3	3
"BGR 436"								
1. Untreated control	-	-	-	-	-	-	-	-
2. Stomp Aqua – 4.00 L ha^{-1}	2	1	1	1	2	1	1	1
3. Butizan 400 SC – 2.50 L ha^{-1}	4	2	1	1	4	2	1	1
4. Lontrel 300 SL – 0.40 L ha^{-1}	5	4	2	2	5	4	2	2
5. Galera Super – 0.20 L ha^{-1}	6	4	3	2	6	4	3	2

In general, the Italian camelina genotype "CAM 265" was more sensitive to foliar-applied herbicides. The lowest phytotoxicity for the three camelina genotypes after the treatment with Stomp Aqua – 4.00 L ha^{-1} was found. In both experimental years, phytotoxicity score 2 was detected on the 7th day after germination, with symptoms expressed in a slight suppression of the growth of plants that were sown more shallowly. On the following reporting dates, phytotoxic symptoms were not reported and the phytotoxicity was score 1 (healthy plants).

The phytotoxicity recorded on the 7th day after germination of the plants was more severely pronounced for treatment 3 – Butizan 400 SC – 2.50 L ha^{-1} – score 4.

High phytotoxicity was reported after the application of the foliar herbicide Lontrel 300 SL – 0.40 L ha^{-1} and Galera Super – 0.20 L ha^{-1}. Despite the more effective weed control, the yields of all three camelinas were severely reduced by the subsequent herbicide stress after spraying.

In the treatment with Galera Super and Lontrel 300 SL on the 7th day after treatments, a phytotoxicity score of 5 was recorded for the Poland camelina genotype "Czenstochowska", and the Bulgarian camelina landrace "BGR 436". The symptoms of visual phytotoxicity are expressed in plant wilting, suppressing the growth of the vegetation tip and from there slowing down the growth and development of the plants. On the 14th day after treatment, the phytotoxicity decreased to score 4, and the symptoms described above were less pronounced. On the 21st and 28th days, the symptoms of phytotoxicity decreased to score 2. For the camelina "CAM 265", the symptoms of

phytotoxicity were more serious and reached score 6 and score 5, respectively, on the 7th and 14th day after treatment. On the 21st and 28th days, phytotoxic symptoms were evaluated with a score of 3 and 2, respectively. A delay in the entry of plants into the flowering phenophase was reported as well.

After the treatment with Galera Super – 0.20 L $ha^{-1,}$ the phytotoxicity was generally higher than that of the treatment with Lontrel 300 SL – 0.40 L ha^{-1}. On the 7th day after spraying, a phytotoxicity score of 6 for the Poland camelina genotype "Czenstochowska" and the Bulgarian landrace "BGR 436" was recorded. The symptoms of visual phytotoxicity were stronger stunting of the plants, stronger suppression of the growth of the vegetative tip, and growth retardation. On the 14th day after treatment, the phytotoxicity diminished to a score 4, and the symptoms described above were lowered. On the 21st and 28th days, the symptoms of phytotoxicity decreased to a score of 3 and 2, respectively. In *Camelina* genotype "CAM 265", the symptoms of phytotoxicity were more severe and reached scores 6 and 5 respectively on the 7th and 14th day after application. On the 21st and 28th days, the phytotoxic symptoms were evaluated with a score of 3. A greater delay in the entry of plants into the flowering stage was found as well.

Plant height in camelina varies from 65 to 110 cm and is strongly influenced by external factors such as growing season, nutritional regime, rainfall, etc. (Berti et al., 2011). Researchers around the globe have reported a disturbance in plant growth after plants' exposure to herbicidal stress (Varshney et al., 2015; Neshev et al., 2020, Marques et al., 2021).

It was re-confirmed in the present study where all three camelina landraces were influenced by the herbicidal treatments. The plants treated with Lontrel 300 SL and Galera Super led to an extension of the vegetation and elongation of the plants (Table 3).

Table 3. Plant height at the end of the vegetation (cm)

Treatments/Year	2022	2023	Average for the period
"Czenstochowska"	cm	cm	cm
1. Untreated control	69.40 bc	65.70 b	67.55
2. Stomp Aqua – 4.00 L ha^{-1}	70.70 bc	65.30 b	68.00
3. Butizan 400 SC – 2.50 L ha^{-1}	66.60 c	69.90 ab	68.25
4. Lontrel 300 SL – 0.40 L ha^{-1}	78.90 a	76.20 a	77.55
5. Galera Super – 0.20 L ha^{-1}	74.20 ab	70.60 ab	72.40
"CAM 265"	cm	cm	cm
1. Untreated control;	69.20 b	69.70 ab	69.45
2. Stomp Aqua – 4.00 L ha^{-1}	63.60 b	65.60 bc	64.60
3. Butizan 400 SC – 2.50 L ha^{-1}	69.80 b	67.30 abc	68.55
4. Lontrel 300 SL – 0.40 L ha^{-1}	76.50 a	72.30 a	74.40
5. Galera Super – 0.20 L ha^{-1}	69.40 b	73.40 a	71.40
"BGR 436"	cm	cm	cm
1. Untreated control	62.60 b	61.60 b	62.10
2. Stomp Aqua – 4.00 L ha^{-1}	66.30 ab	63.10 b	64.70
3. Butizan 400 SC – 2.50 L ha^{-1}	67.30 ab	73.40 a	70.35
4. Lontrel 300 SL – 0.40 L ha^{-1}	71.10 a	75.80 a	73.45
5. Galera Super – 0.20 L ha^{-1}	66.60 ab	62.20 b	64.40

[a] Means with different letters are with proved differences according to Duncan's Multiple Range test ($p < 0.05$).

The genotypes selected for evaluation in this study are relatively short and respond differently to the applied herbicides. The stems are weakly hairy, well-branched, become woody when mature, and no lodging was observed during both experimental years.

Table 3 presents the change in the height of the plants of the studied camelina genotypes under the influence of the applied herbicidal products and compared with the untreated control.

On average for the period, the lowest plants for the untreated control from genotypes "Czenstochowska" and "BGR 436" - 67.55 and 62.10 cm, respectively were measured. In camellia genotype "CAM 265", the lowest plants were measured in variant 2 (Stomp Aqua – 4.00 L ha^{-1}) - 64.60 cm average for the period. The herbicidal stress caused by the foliar treatment with Lontrel 300 SL – 0.40 L ha^{-1} and Galera Super – 0.20 L ha^{-1} (variants 4 and 5) led

to an extension of the vegetation. Plants of the three camelina genotypes treated with these herbicidal products were taller than those of the other variants. The only exception is variant 5 of camelina genotype "BGR 436", where the plants are shorter.

The number of primary branches plant^{-1} at the end of the vegetation was influenced by the herbicidal treatments as well. The number of branches was higher for the soil-applicable herbicides Stomp Aqua and Butizan when compared with Lontrel 300 SL and Galera Super. In Table 4 the established results for the number of primary branches plant^{-1} in the investigated camelina genotypes are shown. On average for the period, the plants of variant 3 (Butizan 400 SK – 250 ml/da) for genotypes "Czenstochowska" and "CAM 265", as well as variant 2 (Stomp Aqua – 4.00 L ha^{-1}) for genotype "BGR 436" have the highest number of branches – 12.20, 13.10 and 9.80, respectively. The plants treated with Galera Super – 0.20 L ha^{-1} (variant 5) had the lowest number of primary branches - 6.95, 5.70, and 4.45 on average for the period for the three grown camelina genotypes respectively.

Table 4. Number of primary brunches plant^{-1} at the end of the vegetation

Treatments/Weeds/Year	2022	2023	Average for the period
"Czenstochowska"	plant^{-1}	plant^{-1}	plant^{-1}
1. Untreated control	11.10 b	7.00 b	9.05
2. Stomp Aqua – 4.00 L ha^{-1}	10.30 b	7.00 b	8.65
3. Butizan 400 SC – 2.50 L ha^{-1}	14.90 a	9.50 a	12.20
4. Lontrel 300 SL – 0.40 L ha^{-1}	10.50 b	7.50 b	9.00
5. Galera Super – 0.20 L ha^{-1}	6.80 c	7.10 b	6.95
"CAM 265"	plant^{-1}	plant^{-1}	plant^{-1}
1. Untreated control;	9.40 b	9.40 b	9.40
2. Stomp Aqua – 4.00 L ha^{-1}	9.10 bc	9.80 b	9.45
3. Butizan 400 SC – 2.50 L ha^{-1}	13.00 a	13.20 a	13.10
4. Lontrel 300 SL – 0.40 L ha^{-1}	9.00 bc	12.60 a	10.80
5. Galera Super – 0.20 L ha^{-1}	6.40 c	5.00 c	5.70
"BGR 436"	plant^{-1}	plant^{-1}	plant^{-1}
1. Untreated control	7.20 b	9.50 a	8.35
2. Stomp Aqua – 4.00 L ha^{-1}	9.70 a	9.90 a	9.80
3. Butizan 400 SC – 2.50 L ha^{-1}	7.00 b	6.20 b	6.60
4. Lontrel 300 SL – 0.40 L ha^{-1}	7.70 ab	10.00 a	8.55
5. Galera Super – 0.20 L ha^{-1}	4.30 c	4.60 c	4.45

[a] Means with different letters are with proved differences according to Duncan's Multiple Range test ($p < 0.05$).

Studies of different genotypes showed a strong variation in the silique number formed by a plant. The silique number correlates positively with nitrogen fertilization and sowing density (Czarnic et al., 2018). On average, about 60 to 115 boxes develop on one plant, changing their colour from green to yellow-reddish when ripe and then drying to full maturity (Jankowski et al., 2019). In a previous experiment with oilseed rape, Neshev et al. (2021) recorded a decrease in the silique number developed from a plant after imitation of herbicide drift that caused stress to the crop. In the present experiment with camelina varieties, there was silique number per plant decreased as well (Table 5). The results, calculated for the number of pods from one plant, are presented in Table 5. On average for the period, the plants from variant 3 (Butizan 400 SC – 2.50 L ha^{-1}) at genotype "Czenstochowska" had the highest number of siliques - 361.90. Plants with genotypes "CAM 265" and "BGR 436" formed the highest silique number plant^{-1} after the application of Stomp Aqua - 4.00 L ha^{-1} (variant 2) - 236.95 and 222.70, respectively. The plants treated with Lontrel 300 SL - 0.40 L ha^{-1} (variant 4) had the lowest silique number plant^{-1} - 99.3, 98.60, and 94.95 on average for the period for genotypes "Czenstochowska", "CAM 265", "BGR 436" respectively. Plants from the untreated control formed a greater number of pods per plant compared to variant 4 (Lontrel 300 SL).

Table 5. Number of siliques plant^{-1} at the end of the vegetation

TreatmentsYear	2022	2023	Average for the period
"Czenstochowska"	plant^{-1}	plant^{-1}	plant^{-1}
1. Untreated control	255.20 b	195.80 b	225.50
2. Stomp Aqua – 4.00 L ha^{-1}	367.30 a	187.20 bc	277.25
3. Butizan 400 SC – 2.50 L ha^{-1}	419.00 a	304.80 a	361.90
4. Lontrel 300 SL – 0.40 L ha^{-1}	91.10 c	107.50 c	99.30
5. Galera Super – 0.20 L ha^{-1}	178.00 b	188.60 bc	183.30
"CAM 265"	plant^{-1}	plant^{-1}	plant^{-1}
1. Untreated control;	215.90 b	182.70 a	199.30
2. Stomp Aqua – 4.00 L ha^{-1}	328.80 a	145.10 c	236.95
3. Butizan 400 SC – 2.50 L ha^{-1}	304.80 a	142.90 c	223.85
4. Lontrel 300 SL – 0.40 L ha^{-1}	57.20 c	140.00 c	98.60
5. Galera Super – 0.20 L ha^{-1}	254.10 ab	165.30 b	209.70
"BGR 436"	plant^{-1}	plant^{-1}	plant^{-1}
1. Untreated control	156.10 b	172.70 a	164.40
2. Stomp Aqua – 4.00 L ha^{-1}	276.40 a	169.00 a	222.70
3. Butizan 400 SC – 2.50 L ha^{-1}	194.00 ab	144.00 b	169.00
4. Lontrel 300 SL – 0.40 L ha^{-1}	67.20 c	122.70 c	94.95
5. Galera Super – 0.20 L ha^{-1}	188.00 b	170.80 a	179.40

[a] Means with different [letters] are with proved differences according to Duncan's Multiple Range test ($p < 0.05$).

The herbicidal stress may lead to a severe yield decrease in crops (Deeds et al., 2006; Webster et al., 2016; Neshev et al., 2021). All three landraces had lower yields after experiencing herbicidal damages (Table 6). The lowest yield plant^{-1} for variant 5 (Galera Super – 0.20 L ha^{-1}) and for the three camelina genotypes – 0.64 g ("Czenstochowska"), 0.64 g ("CAM 265"), and 0.53 g ("BGR 436") average for the study years was reported. In variant 4 (Lontrel 300 SL – 0.40 L ha^{-1}) the yields from one plant were slightly higher when compared to those of variant 5. On average for the two research yes, the plants of variant 2 (Stomp Aqua – 4.00 L ha^{-1}) stand out with the highest yields for the three camelina genotypes - 2.20 g ("Czenstochowska"), 1.66 g ("CAM 265"), and 1.77 g ("BGR 436"). The seed yields of the control plants averaged 1.50 g ("Czenstochowska"), 1.22 g ("CAM 265"), and 1.16 g ("BGR 436").

Table 6. Seed yield plant^{-1}, g

Treatments/Weeds/Year	2022	2023	Average for the period
"Czenstochowska"	plant^{-1}	plant^{-1}	plant^{-1}
1. Untreated control	1.63 b	1.36 a	1.50
2. Stomp Aqua – 4.00 L ha^{-1}	3.07 a	1.33 a	2.20
3. Butizan 400 SC – 2.50 L ha^{-1}	1.66 b	0.91 ab	1.29
4. Lontrel 300 SL – 0.40 L ha^{-1}	0.82 c	0.93 ab	0.88
5. Galera Super – 0.20 L ha^{-1}	0.54 c	0.74 b	0.64
"CAM 265"	plant^{-1}	plant^{-1}	plant^{-1}
1. Untreated control;	1.56 b	0.88 b	1.22
2. Stomp Aqua – 4.00 L ha^{-1}	2.58 a	0.74 bc	1.66
3. Butizan 400 SC – 2.50 L ha^{-1}	1.60 b	0.85 b	1.23
4. Lontrel 300 SL – 0.40 L ha^{-1}	0.96 c	1.01 a	0.99
5. Galera Super – 0.20 L ha^{-1}	0.74 c	0.54 c	0.64
"BGR 436"	plant^{-1}	plant^{-1}	plant^{-1}
1. Untreated control	1.47 b	0.85 ab	1.16
2. Stomp Aqua – 4.00 L ha^{-1}	2.48 a	1.06 a	1.77
3. Butizan 400 SC – 2.50 L ha^{-1}	1.24 b	0.66 ab	0.95
4. Lontrel 300 SL – 0.40 L ha^{-1}	0.93 c	0.67 ab	0.80
5. Galera Super – 0.20 L ha^{-1}	0.54 c	0.52 b	0.53

[a]Means with different [letters] are with proved differences according to Duncan's Multiple Range test ($p < 0.05$).

CONCLUSIONS

More efficient weed control was found from the foliar applications of Galera Super and Lontrel 300 SL, but higher phytotoxicity was found after the application of these herbicides. The camelina genotype "CAM 265" was the most sensitive to the herbicide applications. The lowest plants were measured in the untreated control from genotypes "Czenstochowska" and "BGR 436". The plants treated with Butizan 400 SC developed the highest number of branches for genotypes "Czenstochowska" and "CAM 265", and the plants of genotype "BGR 436" developed the highest number of branches after treatment with Stomp Aqua. The lowest number of branches was counted for the plants treated with Galera Super. The plants of genotype "Czenstochowska" formed the highest number of siliques where Butizan 400 SC was sprayed. The plants in genotypes "CAM 265" and "BGR 436" formed had the highest silique number after the application of Stomp Aqua. The lowest seed yields per plant were recorded when Galera Super was applied, and the highest seed yield per plant - when Stomp Aqua was sprayed.

ACKNOWLEDGEMENTS

The research was financially supported by project *CARINA: Carinata and camelina boosting the sustainable diversification in agricultural production systems* (№ 101060558), funded from the European Union's Horizon Europe research and innovation programme.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

INVESTIGATING THE IMPACT OF SPECIFIC TECHNOLOGIES ON THE PRODUCTION AND QUALITY OF THE AUTUMN BARLEY CROP

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Abstract

The purpose of this work was to evaluate the impact of soil tillage, sowing period and mineral fertilization (NPK) on the quantity and quality of the seed harvest, in the specific climate and soil conditions of the Arad Plain, more precisely in the chernozem microzone of Nădlac, Arad County. The use of ploughing in association with disc harrow determined a small and insignificant increase in production compared to plots where only disc passes were applied. The results obtained demonstrate the superiority of the tillage variants without furrow turning, compared to the classic tillage system by ploughing. Sowing in the period 25-30.IX and staggered in 06-10.X, did not attract, on average over the two years, any particular differences between the variants. Mineral fertilization (NPK) of the soil plays an essential role in optimizing the plant`s nutrition requirements and achieving increased production. In addition, the tillage system and fertilization also influence soil fertility in the long term, a significant impact on other components of the environment. The variety of barley taken into cultivation was Melia.

Key words*: technological links, barley, production, quality indices.*

INTRODUCTION

Barley (*Hordeum vulgare* L.) is one of the oldest cereal crops, having a special economic and agricultural importance both nationally and globally. Barley is a winter crop well known for its small-seeded grains and self-pollinating characteristics. Flour derived from barley grains plays a crucial role in numerous processed foods, contributing to their taste and nutritional value (Kaur et al., 2024). Due to its adaptability to various climatic conditions, barley is widely cultivated, being used in the food industry, fodder and beer production (Boanta et al., 2020), it is also used in its form of pearl barley and as a coffee substitute in the human diet, it is a raw material for the production of beer, glucose and alcohol (Tarawneh et al., 2021; Gupta et al., 2010). It is also an important source of feed for animals that consume it in the form of cereals, green feed, straw and hay (Badea and Wijekoon, 2021).

From these, winter barley is an appreciated crops by farmers due its ability to adapt to less favourable environmental conditions and also give high and economically efficient yields. The barley grain weight (GW) along with grain size are universally considered to be closely correlated with the level of technological quality indicators required by the malt and beer industry. Grain weight is first trait used to classify the barley seed quality (Vasilescu et al., 2020; Leonte et al., 2023). Drought and heat stress substantially impact plant growth even the productivity of barley culture. When subjected to drought or heat stress, plants exhibit reduction in growth resulting in yield losses (Mahalingam et al., 2022).

In Romania, barley occupies a significant place in the agricultural sector, being grown both for domestic consumption and for export (Cristea et al., 2024).

The area cultivated with barley varies annually, but remains at a considerable level, due to the constant demand from the malt industry and the livestock sector.

Barley has a short vegetation period and good drought resistance, which makes it suitable for Romania's climate, especially in areas with low rainfall. It is grown in both autumn and spring variants, each having specific uses. Autumn barley is preferred for feed production, due to its high yield, while spring barley is essential in the beer industry, being used to obtain malt.

Economically, Romania is an important producer and exporter of barley in the European Union, with markets in the Middle East and Asia. The increase in foreign demand for Romanian barley contributes to the development of the agricultural sector and to the balancing of the trade balance. Through its agricultural, economic and food value, barley remains an essential crop for Romania and for the whole world.

A unique blend of macro and micronutrients makes barley an important industrial substrate for commercial use (Boukid, 2024; Raj et al., 2023; Lazaridou et al., 2022).

Barley consists of complex carbohydrates (80%), proteins (11.5-14.2%), lipids (4.7-6.8%), β-glucan (3.7-7.7%) and ash (1.8-2.4%). Starch is the most prevalent nutrient in barley, accounting for 70% of the total dry weight of the kernel (Asare et al., 2011; Baik and Ullrich, 2008; Li et al., 2001).

Barley is not just a cereal crop, but a vital resource for human health, food security and environmental sustainability (Alionte, 2006).

Under current climate change conditions, water stress is becoming a major limiting factor for cereal production. The qualitative variation of winter barley grains under water-restrictive conditions, highlights the significant differences between genotypes and their potential to translocate assimilates into grains during moisture deficit. These results provide an important starting point for the selection of varieties adapted to difficult pedoclimatic conditions (Vasilescu et al., 2023).

Continued scientific research and innovations in agriculture and the food industry will further strengthen the importance of this cereal within global food systems.

MATERIALS AND METHODS

The purpose of this work was to evaluate the effects of tillage, sowing time and mineral fertilization (NPK) of the soil on the quantity and quality of the seed yield when cultivating the plant in climatic and soil conditions in the Arad Plain.

The objective of the research refers to: establishing and implementing the cultivation technology; evaluation of production according to tillage; evaluation of production according to sowing time; evaluation of production according to fertilization; determination of weight attributes (TKW, HW); determination of crude protein content; determination of starch content.

The cultivation technology, a factor of agricultural production, has an essential role in creating the conditions for the growth and development of the plant, along with the natural environmental factors (soil, climate) and in making the production process more efficient, and the improvement of agrotechnical cultivation methods is always a concern for agricultural research and practice, an effort to which these researches are modestly added.

The study focused on the technological sequences that underlie the optimization of the plant's cultural requirements, against the background of the soil and climate, and whose suitability and quality of application largely depends on the development of the plant and the level of production achieved, in addition, the tillage system and fertilization also have an impact on the evolution of soil fertility and other environmental components over time.

The aim of the study is to find solutions for the expansion of crop barley, given that this species is a rich source of starch and protein with applicability in various economic sectors, such as the beer industry or animal feed (Ungureanu et al., 2019; Ungurean et al., 2021).

The experiment organized on the gleic chernozem was monofactorial type, with the following variants: V1 – plow + disc harrow, V2 – disc harrow × 2 and V3 – scarifier + disc harrow.

In order to ensure fertilization levels, simple fertilizers based on nitrogen, phosphorus and potassium were administered, namely: ammonium nitrate (33.5%) 269 kg ha^{-1}, superphosphate (46%) 130 kg ha^{-1} and potassium salt (50%) 160 kg ha^{-1}.

In 2022, January - March: the period was characterized by low temperatures, Figure 1, specific to the cold season, with moderate precipitation ensuring soil moisture; April - June: temperatures increased germination and vegetative development; July - September: high temperatures were recorded, with episodes of drought, which led to variations in precipitation; October - December: temperatures began to decrease; the rainfall recorded during

this period was variable, influencing the preparation for the cold season.

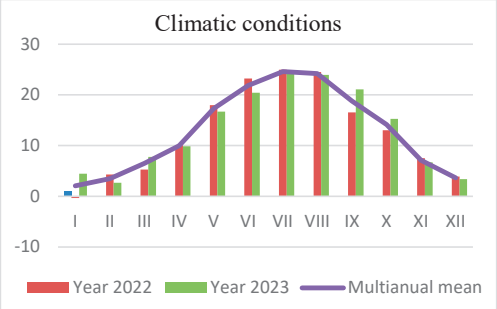


Figure 1. Temperatures regime during the experimentation period, 2022-2023

In 2023, January - March: data showed similar trends to the previous year, with temperature and precipitation values according to specific seasonality; April - June: temperatures and precipitation showed significantly favorable trends for barley, reaching averages between 15°C and 25°C; precipitation, Figure 2, was constant during this period, being favorable for growth, similar to previous years; July - September: periods of extreme heat were recorded, with sporadic precipitation that could negatively influence the harvest; October - December: the gradual cooling of the weather was accompanied by variable precipitation.

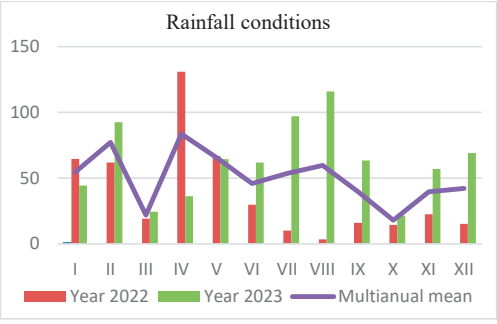


Figure 2. Rainfall regime during the experimentation period, 2022-2023

The fertilization levels studied were obtained by applying ammonium nitrate, superphosphate and potassium salt (kg ha^{-1}, etc.). The precursor plant was rapeseed crop. The barley variety taken in cultivation was MELIA, varieties belonging to the LIDEA company.

The experiment was located in the city of Nădlac, Arad County, on a chernozem - type soil.

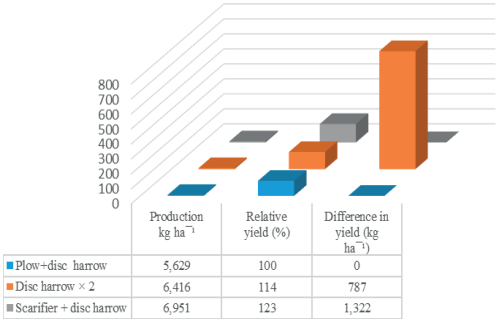
RESULTS AND DISCUSSIONS

Results obtained demonstrate the superiority of the tillage variants without furrow turning, compared to the classic tillage system by ploughing.

Results of the research on the influence of the basic tillage system on the harvest

In Figure 3, the summary results on the average barley productions according to tillage in the 2022-2023 experimental cycle are presented. The use of ploughing in association with disc harrow determined a small and insignificant increase in production compared to plots where only disc harrow passes were applied. The largest harvests were obtained in the variant in which the scarification of the soil + 2 × disc harrow was carried out.

The yield difference between the scarified variant and the control variant shows 1,322 kg ha^{-1}, it is statistically ensured as very significant.



	Production kg ha^{-1}	Relative yield (%)	Difference in yield (kg ha^{-1})
Plow+disc harrow	5,629	100	0
Disc harrow × 2	6,416	114	787
Scarifier + disc harrow	6,951	123	1,322

Figure 3. Summary of average barley yields by tillage in 2022-2023

Results of the research on the influence of the sowing time on harvest

In Figure 4 is presented the timing of sowing influences the evolution of plants by determining the time available for the normal stages of growth in autumn, including twinning and hardening before winter.

The sowing in the period 25-30.IX and staggered in 06-10.X, did not attract, on average over the two years, any particular differences between the variants, the average production value being 5,704 kg ha^{-1} at the first

time and 6,314 kg ha^{-1} at the second sowing period.

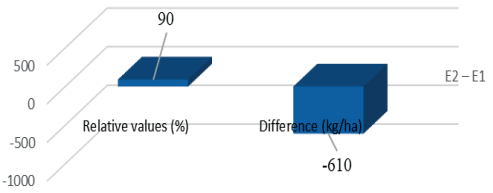


Figure 4. Summary of average barley yields by sowing time in 2022-2023

Sowing in the first days of October allows to avoid too advanced development of autumn vegetation and reduce the risk of attack by aphids and cicadas, which can transmit the dwarf and yellowing virus to barley. Overall, against the background of the climatic conditions of 2022-2023, sowing at the end of September led to a distinctly significant increase in production, by 610 kg ha^{-1}.

Results of the research on the influence of fertilization on the harvest

Mineral fertilization (NPK) of the soil plays an essential role in optimizing the plant's nutrition requirements and achieving increased production. It is noted in Figure 5 that the fertilizers applied, in the mentioned doses, strongly influenced the harvest in the two experimental years.

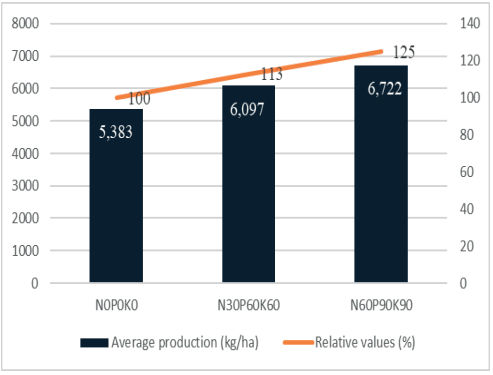


Figure 5. Synthesis of average barley yields for different fertilization treatments in the 2022-2023 experimental cycle

By fertilizing with N30, the average harvest increased by 13% compared to the control variant, registering a harvest difference of 714 kg ha^{-1}. The doubling of the nitrogen dose to N60 amplified the value of the harvest increase to 25%, returning a harvest difference compared to the control variant of 1,339 kg ha^{-1}. In both variants, the harvest results were statistically assured as very significant.

Results of the research on hectoliter mass values by sowing time

The hectoliter mass of barley is a measure of the density of barley grains and is a relevant indicator in assessing the quality and commercial value of this cereal crop. The term 'hectoliter mass' refers to the mass of barley grains expressed in kilograms per hectoliter (kg/hl), i.e. the amount of barley occupying a volume of one hectoliter. This measure provides significant information about the uniformity, density and usability potential of barley grains in various applications, including beer production, bakery and feed. Hectoliter mass is influenced by several factors, including the size, shape, weight, and density of barley grains. Hectoliter mass is often used as an important criterion in assessing the quality of barley grains. The higher the hectoliter mass of barley grains, the higher their density, reflecting a higher dry matter content. Considering the cumulative effect of the sowing season, in the agricultural year 2021-2022 (Table 1) the average values of the HM were recorded with the limits of 62.2 kg in the case of the establishment of the crop at the end of September and 62.5 kg in the case of sowing in the first days of October, against the background of a non-significant variation between the two years.

Table 1. Average value of HM for barley for sowing times in 2021-2022

Sowing	HM (kg/hl)		Relative values (%)	Difference (kg/ha)	Signification
$E_2 - E_1$	62.5	62.2	100	0.3	
DL 5% = 0.55 KG/HL DL 1% = 0.83 KG/HL DL 0.1% = 1.33 KG/HL E1 – 25-30 IX 2022; E2 – 5-10 X 2022					

In the context of the 2022-2023 agricultural season, the overall analysis of the data revealed in Table 2 indicates a minor influence on the hectoliter mass following the delay in sowing from the end of September to the beginning of October. This delay generated a variation of only 0.2 kg in the hectoliter mass, with a negligible difference in this characteristic.

Specifically, the detailed analysis of the results suggests that the delay in sowing during the mentioned period did not have a significant impact on the quality of the crop's hectoliter mass. It is important to emphasise that this finding is supported by a minimum variation of only 0.2 kg, which can be considered statistically insignificant.

This finding is of interest in the context of planning and managing the agricultural season, providing practical information for farmers and researchers.

In conclusion, the scientific analysis of the data from the agricultural year 2022-2023, on the effect of the sowing delay on the hectoliter mass, indicates a low influence, represented by an insignificant variation of 0.2 kg. It makes valuable contributions to the understanding of the relationships between the sowing period and crop qualities, having significant practical implications for the efficient management of agricultural resources.

Table 2. Average value of HM in barley for sowing times in 2022-2023

Sowing	HM (kg/hl)		Relative values (%)	Difference (kg/ha)	Signification
$E_2 - E_1$	61.6	61.4	99	0.2	
DL 5% = 0.74 kg/hl DL 1% = 1.17 kg/hl DL 0.1% = 2.14 kg/hl E_1 – 25-30 IX 2022; E_2 – 5-10 X 2022					

Values of the relative of thousand kernel weight grains (TKW) according to the sowing time of the barley crop.

The determination of this physical property makes it possible to appreciate, on the one hand, the amount of food available to the sprouts, and on the other hand, the behavior of the different varieties in the given growing conditions that influence the size of the harvests. Dependent on TKW is also the vigor of the sprouts, which conditions the growth rate of the plants and ultimately the harvest. This physical seed attribute is part of the formula for determining the amount of seeds needed for sowing ha^{-1} and the formula for approximating the probable harvest. The results of the analyses highlight (Figure 6) a significant correlation between the TKW value and the climatic conditions in the context of the two sowing seasons. In the agricultural year 2021-2022, characterized by a rainfall deficit, the TKW recorded an average value of 39.9 g. This decrease can be attributed to drought conditions, which affected plant development and negatively influenced grain weight. On the other hand, in the agricultural year 2022-2023, in which climatic conditions were favorable, with an adequate rainfall intake, MMB recorded a significant increase, reaching 46.2 g. This improvement of 6.3 g indicates a positive influence of the wet environment on the process of grain formation.

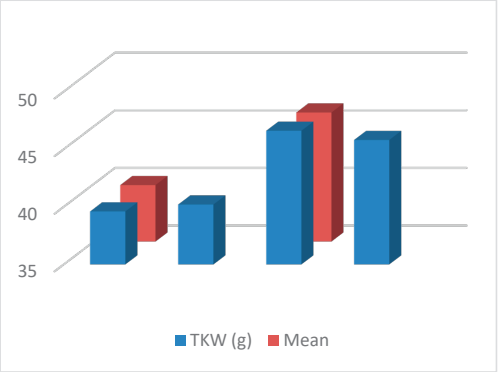


Figure 6. Results of the TKW determinations in experimental years according to the sowing period

Results of chemical analyses

From the data of the analyzes in Figure 7, it appears that in 2021-2022, in all variants, the protein content was higher than in the experimental year 2022-2023, which is due to the drought installed between May and June, which favored the accumulation of protein to the detriment of starch.

In the agricultural year 2022-2023, in the months of May - June, higher amounts of precipitation fell, which caused a decrease in protein content and a favoring of starch content. Nitrogen fertilizers, on average over the two experimental years, favored the protein content, which increased compared to the control variant, from 13.5% to 14.6% in the N30 variant and to 15.6% in the N60 variant. On average over the two experimental years, the fertilization doses shown that the valorization of nitrogen fertilizers, at this level, is motivated even in the conditions of the fertile soil on which the experiments were carried out.

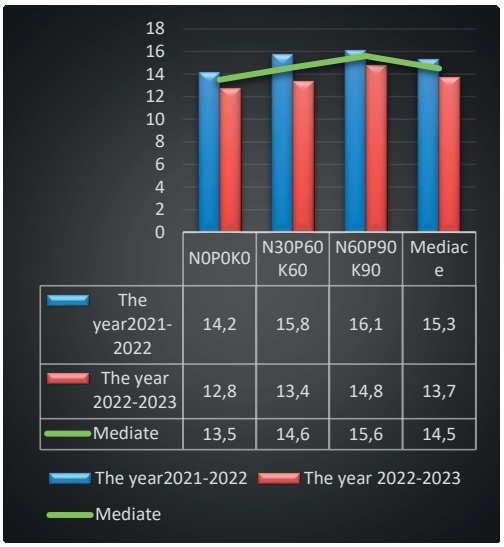


Figure 7. Variation in crude protein content depending on fertilization

Variation of starch content depending on fertilization

The results of the analyzed data present in Figure 8, show that in the drier year of the grain formation and filling period 2021-2022, the starch content was lower than the content of 2022-2023, which is explained by the higher amount of rainfall.

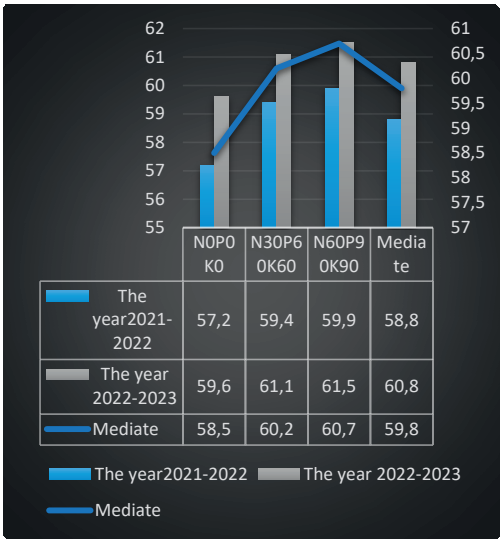


Figure 8. Starch content % by fertilization

CONCLUSIONS

The high yields of barley for grains in the last decades, of 8-10 t ha^{-1} under appropriate technology conditions, motivate the expansion of this crop. The shorter vegetation period than wheat, the higher drought resistance compared to wheat, makes it possible that after the barley harvest, successive crops can be established that provide increases that increase the profit per unit area and keep the soil covered with vegetation until autumn.

Basic tillage experienced in the conditions of the gleic chernozem in Nadlac have demonstrated that the traditional ploughing work, by which the furrow is turned over, with negative effects on the loss of moisture from the soil and disturbance of the activity of microorganisms, can be replaced by superficial works without turning the furrow. The harvest increase obtained through the double tillage system ensured the increase of the harvest by 17% compared to the ploughing work option.

The highest harvest increase was obtained in the variant in which scarification was carried out and a double disc harrow of 31% in the period 2021-2022 and by 12% in the variant by double disc harrow and 17% in the variant scarified + double disc harrow in 2022-2023.

The research on the influence of the sowing time on the harvest varied between the two years due to the rainfall regime during the land preparation period and sowing in autumn.

On average, over the two years, research has highlighted the first decade of October as optimal.

In conclusion, the results of the experiment highlight that proper fertilization, especially with nitrogen, phosphorus and potassium, significantly influences crop production.

Under the analyzed conditions, the variants fertilized with N30P60K60 contributed to the increase of production with very significant differences of 18% and 31% in the N60P90K90 variant compared to the control variant N0P0K0 in 2021-2022 and with increases of 9% in the N30P60K60 variant and 20% in the N60P90K90 variant in the experimental year 2022-2023, represented effective options for increasing yield, stressing the need for proper fertilizer management in modern agriculture,

with a significant impact on the sustainability and profitability of agricultural production.

In conclusion, the scientific analysis of the data from the agricultural year 2022-2023, on the effect of the sowing delay on the hectoliter mass, indicates a very low influence, represented by an insignificant variation of 0.2 kg. It makes valuable contributions to the understanding of the relationships between the sowing period and crop qualities, having significant practical implications for the efficient management of agricultural resources.

The experimental results obtained in the two years proved that the climatic deviations in the vegetation period greatly influence the results obtained, which is why another year of research is required and only by analyzing the synthesis of the experimental cycle can conclusions be drawn that can be transformed into recommendations for growers.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

RESEARCH ON THE INFLUENCE OF FERTILIZATION ON *Lavandula angustifolia* Mill. SPECIES IN THE ORGANIC CULTURE OF THE BUFTEA AREA (ILFOV)

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Abstract

Lavandula angustifolia Mill., part of the Lamiaceae family, is a medicinal and aromatic plant used in various industries, such as the pharmaceutical and cosmetic ones. This paper discusses aspects concerning the growth and development of Lavandula angustifolia Mill. (the Emilia and George 90 varieties) when fertilization products approved for organic agriculture are applied. In terms of floral stem and inflorescence development, it is observed that the George 90 variety has larger dimensions than the Emilia variety. The George 90 variety had a floral stem length of 60.25 cm, whereas the Emilia variety measured 58.45 cm. From the perspective of the increase in length (cm) of the inflorescence, it was found that the George variety had a longer inflorescence (13.23 cm) compared to the Emilia variety (8.18 cm).

Key words*: fertilization, Lavandula, biometric parameters.*

INTRODUCTION

Lavandula angustifolia Mill. (lavender, also known as *Lavandula officinalis*) is part of the Lamiaceae family (formerly Labiatae) and is a woody (aromatic) shrub to the Mediterranean Mountains (around the Mediterranean Sea - Ghavani et al., 2022). It is grown in Northern and Eastern Africa, Southern Europe, the United Kingdom, the United States, Southwestern Asia, Australia, and even in India (Basch et al., 2004; Pleguezuelo et al., 2009). Namdeo (2018) mentions that there are hundreds of plant genera used in herbal remedies as well as medicines (traditional and folk medicine) worldwide.

Medicinal and aromatic plants (including *Lavandula angustifolia* Mill.) are an important source of nutrients and natural remedies, as noted by Bouzid (2023).

According to Ghavani (2022), Lavandula species (lavender) are among the medicinal plants that aid in stress management, encompassing over 40 species and hundreds of hybrids. Notable examples include: *Lavandula angustifolia* Mill., a frost-resistant species with many decorative flower varieties, and the most cultivated of the four species (Açıkgöz, 2024); *Lavandula stoechas* (formerly known as *Lavandula vera*), a tall plant with gray-green foliage, late blooming, but with a strong characteristic scent; *Lavandula latifolia* (French lavender); and *Lavandula intermedia*, which is actually a hybrid between *Lavandula latifolia* and *Lavandula angustifolia* (Açıkgöz et al., 2024; Ghavani et al., 2022).

Cardoso et al. (2022) mention that management methods, including biological, mechanical, and molecular approaches, have been developed with the aim of replacing all synthetic products used as fertilizers and pesticides.

Genetically modified microorganisms will become more efficient biofertilizers or biocontrol agents in the future, with a favorable effect on plant productivity (Pole et al., 2022).

The author Quarashi (2024) mentions that ornamental medicinal plants are of particular economic importance because they are used both for landscaping (remediating degraded lands) and for phytomanagement of waste heaps, providing long-term delivery of ecosystem goods and services. They are also used in the cut flower industry, while also

possessing medicinal (therapeutic) and even phytochemical values (Pandey et al., 2004).
Medicinal plants have been widely used since ancient times for their medicinal and culinary properties, in the pharmaceutical and cosmetic industries as well (hair-care products, body products, face creams, etc.), and they also represent an important group of species containing volatile compounds. These plants can be successfully cultivated on degraded or contaminated soils (Greff et al., 2023; Pandey et al., 2024; Shakoor et al., 2024).
The effects of fertilization on the microbial properties of the soil greatly depend on the product and the cultivated species (Zubek et al., 2012).
Fertilizations with phosphorus and potassium influence the physiological processes (the intensity of photosynthesis and transpiration) of commercial lavender (*Lavandula angustifolia* Mill.), according to research conducted by Camen et al., 2016.
Organic fertilizers applied to crops induce positive effects on the organic carbon, nitrogen, and phosphorus content in the soil, according to research conducted by Morugán-Coronado et al., 2020.
Long (2018) mentions that there are over 85,000 ornamental plants worldwide, and these are cultivated in various regions of the planet (Earth), many of which are commercially available on the global market due to their popularity among consumers (Long et al., 2024).
The medicinal plants belonging to the Lamiaceae family (*Lavandula dentata, Mentha spicata, Origanum vulgare, Rosmarinus officinalis* and *Thymus vulgaris*) are rich in mineral substances (especially ales in phosphorus, calcium and magnesium) and proteins as well, simultaneously being aknowledged through research done by Bouzid et al., 2023, that metals și pesticides are found in reduces quantities in plants (lesser than the allowed limits).

MATERIALS AND METHODS

The biological material was presented by two varieties belonging to the *Lavandula angustifolia* Mill. species.
The Emilia and George 90 varieties are included in the Official Catalogue of Varieties in Romania.
The experimental variants are the following:
Factor A - variety: a_1 - Emilia, a_2 - George 90;
Factor B - fertilization: b_1 - CONTROL - UNFERTILIZED, b_2 - COMPOST (10 t/ha), b_3 - COMPOST (10 t/ha) + CROPMAX. (1 l/ha), b_4 - CROPMAX (1 l/ha), b_5 - BLACKJAK (2 l/ha) + CROPMAX (1 l/ha), b_6 - BLACKJAK (2 l/ha).
The planting distances were: for the Emilia variety - 100/120 cm, and for the George 90 variety - 100/100 cm.
The experiment was conducted in the Buftea area, Ilfov County. Fertilizations were applied to the plants, both root and foliar, on April 6th, 2023, and May 7th, 2023.
The details of the two varieties are provided in the Table 1.

Tabel 1. Description of the Emilia and George 90 varieties

Name	Emilia variety	George 90 variety
Plant size	Big compared to other lavander species	Big, distinct compared to all lavander species
Foliar system (color)	Green with light gray tints	Green with light gray tints
Leaf margins	No incisions (absent)	No incisions (absent)
Flowering stem	Long	Very long
Spike	Wide, short	Wide, long
Spike form	Cylindrical	Cylindrica
Flower (calyx)	Green-ish	Green-ish
Pubescence (hairs)	Strong	Strong
Corolla	Violet	Violet
Flowering period	Average	Average

Biometric measurements were taken regarding the length of the floral stem (cm) and the length of the inflorescence (cm). The analysis of the results was statistically expressed using the ANOVA program.

RESULTS AND DISCUSSIONS

The power of fertilization on the lengthiness (cm) of the inflorescence

Based on the data presented in Table 2, it results that the greatest influence on the development of the floral stem (length growth) of the Emilia variety was noticed in variant V_3. The statistical analysis (ANOVA) indicates that variant V_3 exhibited a 1.60 cm increase compared to the landmark (unfertilized) in terms of floral stem-length growth for the Emilia variety, representing 102.81%. It is also

noted that between the first measurement (April 6, 2023) and the last fertilization (July 9, 2023), the floral stems grew by 48.55 cm, representing a percentage increase of 590.40%.



a) b) c) d)

Figure 1. Floral stem length (cm) in May (a, b) and June (c, d) 2023;
a) and c) - Emilia variety; b) and d) George 90 variety

Table 2. The effect of fertilization on the floral stem length (cm) (Emilia variety) in 2023

Experimental version	06.04.	23.04.	07.05.	11.06.	09.07.	Difference (cm) and signification	Percentage compared to landmark (unfertilized)	Difference (cm) (09.07-06.04)	Percentage compared to the value (06.04.2023)
V1	9.43	22.48	32.93	44.81	56.85	Ct	100.00	47.42	602.86
V2	9.63	23.28	33.75	45.66	57.81	0.96	101.68	48.18	600.31
V3	9.90	24.61	35.91	46.51	58.45	1.60*	102.81	48.55	590.40
V4	9.83	23.70	34.30	45.95	58.35	1.50*	102.63	48.52	593.59
V5	9.66	23.53	34.20	45.81	57.91	1.06	101.86	48.25	599.48
V6	9.53	23.08	33.58	45.46	57.51	0.66	101.16	47.98	603.46

LSD 5% = 1.29; LSD 1% = 1.77; LSD 0.1% = 2.43

The data that concers the effect of fertilization on the floral stem length (cm) for the George 90 variety is briefly stated in Table 3, in which it is observed that the greatest power on the growth in length of the floral stem was also registered by variant V_3, with a distinction of 1.69 cm compared to the landmark. The statistical resolution demonstrates that all variants logged increase ranging from 0.89 cm (V_6) to 1.69 cm (V_3) contrasting to variant V_1 (landmark - unfertilized). The greatest accumulation in the floral stem length for the *George 90* variety was stated in variant V_4, with a length of 48.88 cm, constituting the distinction between the first fertilization (06.04.2023) and the last fertilization (09.07.2023), which corresponds to a percentage increase of 499.00%. The highest percentage compared to the value from 06.04.2023 was recorded in variant V_6.

Tabel 3. Power of fertilization on the floral stem length George 90 variety, in the year 2023

Experimental version	06.04.	23.04.	07.05.	11.06.	09.07.	Difference (cm) and signification	Percentage compared to landmark (unfertilized)	Difference (cm) (09.07-06.04)	Percentage compared to the value (06.04.2023)
V1	11.58	24.38	33.96	46.26	58.56	Ct	100.00	46.98	505.69
V2	11.78	24.96	34.48	46.98	59.58	1.02	101.74	47.80	505.77
V3	12.20	25.56	35.00	48.06	60.25	1.69*	102.88	48.05	493.85
V4	12.05	25.43	34.88	48.38	60.13	1.57*	102.68	48.08	499.00
V5	11.86	24.96	34.71	47.23	59.86	1.30	102.21	48.00	504.72
V6	11.68	24.78	34.26	49.98	59.45	0.89	101.51	47.77	508.98

LSD 5% = 1.51; LSD 1% = 2.07; LSD 0,1% = 2.84

The disagreement between the first (06.04.2023) and the last fertilization (09.07.2024), displayed in centimeters (cm) and percentage (%).

It is noticed that between the first and last measurements (for both varieties – Emilia and George 90) concerning the growth in length of the floral stem, the further outcome was registered: for the Emilia variety, the largest distinction was recorded in variant V_3, with 1.60 cm, representing a percentage of 102.81%, while for the George 90 variety, the largest difference was also recorded in the same variant (V_3), specifically 1.63 cm, representing a percentage of 102.88%.

The effect of fertilization on the length (cm) of the florescence

Table 4 presents data regarding the influence of fertilization on the growth of the inflorescence in the Emilia variety.

The most noticeable effect of fertilization on the growth of the measurements of inflorescence was registered in variant V_3, with a distinction of 1.67 cm compared to variant V_1 (the unfertilized landmark).

The related statics exhibits that variants V_2-V_5 recorded growths ranging from 0.99 cm (V_2) to 1.47 cm (V_4) in terms of floral stem length.

The largest percentage difference (125.65%) was recorded in variant V_3.

Regarding the George 90 variety, the gathered data about the influence of fertilization on the length of the inflorescence is emerged in Table 5.

The conclusion drawn that the greatest influence of fertilization was recorded in variant V_3, with a growth of 1.32 cm compared to variant V_1 (unfertilized control), representing 111.08% of the landmark (unfertilized).

Thereby, the analysis and statistics show that for all other variants (V_2, V_4, V_6), the growths ranged between 0.27 cm (V_2) and 0.65 cm (V_5) compared to V_1 (unfertilized landmark).



a) b) c) d)

Figure 2. Floral stem length (cm) in May (a, b) and June (c, d) 2023
a) and c) - Emilia variety; b) and d) George 90 variety

Table 4. The influenza of ferilizing on the length (cm) of the inflorescence (Emilia variety), in the year 2023

Experimental version	06.04.	23.04.	07.05.	11.06.	09.07.	Difference (cm) and signification	Percentage compared to landmark (unfertilized)	Difference (cm) (09.07-06.04)	Percentage compared to the value (06.04.2023)
V1	0.31	2.68	3.45	5.08	6.51	Ct	100.00	6.20	2.100,00
V2	0.41	2.86	3.63	5.38	7.50	0.99	115.20	7.09	1.829,26
V3	0.48	3.31	3.76	5.65	8.18	1.67*	125.65	7.70	1.704,16
V4	0.45	3.15	3.75	5.55	7.98	1.47*	122.58	7.53	1.773,33
V5	0.43	3.08	3.70	5.50	7.73	1.22	118.74	7.30	1.797,67
V6	0.40	2.76	3.56	5.21	7.56	1.05	116.12	7.16	1.890,00

LSD 5% = 1.30; LSD 1% = 1.79; LSD 0,1% = 2.45

Additionally, the greatest difference (growth) between the first fertilization (April 6, 2023) and the last fertilization (July 9, 2023) was recorded in variant V_3, with a growth of 12.65 cm.

Tabel 5. The effect of fertilization on the length (cm) of the inflorescence (George 90 variety), in the year 2023

Experimental version	06.04.	23.04.	07.05.	11.06.	09.07.	Difference (cm) and signification	Percentage compared to landmark (unfertilized)	Difference (cm) (09.07-06.04)	Percentage compared to the value (06.04.2023)
V1	0.36	3.61	5.08	9.66	11.91	Ct	100.00	11.55	3.308,33
V2	0.46	3.83	5.53	10.30	12.38	0.47	103.94	11.92	2.691,30
V3	0.58	4.11	6.25	11.36	13.23	1.32*	111.08	12.65	2.281,03
V4	0.55	4.13	6.10	10.90	12.86	0.95	107.97	12.31	2.338,18
V5	0.50	3.88	5.86	10.55	12.56	0.65	105.45	12.06	2.512,00
V6	0.43	3.68	5.40	10.13	12.18	0.27	102.26	11.75	2.832,55

LSD 5% = 1.10; LSD 1% = 1.51; LSD 0,1% = 2.06

The difference between the first determination (April 6th) and the last fertilization (July 9th), expressed in centimeters (cm) and percentage (%).

The largest difference was registered in variant V_3, with 12.65 cm, signifying a percentage of 2,281.03%, while the most insignificant difference was seen in variant V_6 (11.75 cm) compared to variant V_1 (control).

CONCLUSIONS

It is observed that as a result of the treatments applied (compost and the growth biostimulatory Cropmax), both varieties (Emilia and George 90) showed increases in both the floral stem length and the inflorescence length. Thus, the best results regarding the growth in length (cm) of the floral stem were recorded in variant V_3 (Compost+Cropmax) for both studied varieties. Regarding the growth in length (cm) of the inflorescence, it was shown that variant V_3 was also the most efficient. For the Emilia variety, the growth was 1.67 cm, while for the George 90 variety, it was 1.32 cm, compared to variant V_1 (landmark – unfertilized).

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

DENSITY-BASED ASSESSMENT OF *Adonis vernalis* ABUNDANCE IN NATIVE HABITATS

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Abstract

The study investigates the ecological distribution and abundance of Adonis vernalis *in six natural habitats, identifying significant heterogeneity in species abundance. The analysis of Adonis vernalis habitats is an important step in the better understanding of this species place in the community assemblage related to their native environment: Plants of A. vernalis were counted in 25 m^2 small scale plots from six specific habitats. The data was analysed towards a proposal for the potential distribution of plants in relation to a gradual habitat type.: There were identified two distribution scenarios in relation to the potential distribution of the plants in the habitat. Most of the small-scale plots showed an average of 50 plants/25 m^2, while in three of the habitats were identified patches with more than 200 plants/25 m^2. The forecast for plant presence and distribution indicates two possible outcomes - the first one indicates a maximum pick value of 30-40 plants/25 m^2, and the second one shows two potential picks, at 30 plants/25 m^2 and 120 plants/25 m^2. The A. vernalis habitats present non-linear distribution patterns across the analysed small scale-plots, with the alternation of homogeneous with heterogeneous areas. Given its medicinal value and conservation status, we propose sustainable harvesting strategies that align with the ecological characteristics of the species.*

Key words*: grassland, heterogeneity, habitat dissimilarity, small-scale assessment.*

INTRODUCTION

The conservation of plant biodiversity is an urgent issue due to the rapid decline in the distribution of many species of wild vegetation caused by natural resource use, climate change and other factors (Zhumagul et al., 2024). Many of the endangered plants are of real interest both for maintaining biodiversity and for medicinal use or improving agricultural yields (Kardol et al., 2018; Isbell et al., 2011).

The Ranunculaceae family, distributed almost worldwide, is considered one of the essential groups of ancient angiosperms, has a history of about 75 million years according to fossil records (Anderson et al., 2005; Emadzade et al., 2010; Karahan et al., 2022).

The genus *Adonis* L. (*Ranunculaceae*), widespread in temperate areas of Asia and Europe, depending on the taxonomic concept applied includes between 20 and 40 species, frequently 32 herbaceous annual or perennial species (Aneva et al., 2019; Ghorbani et al., 2008; Karahan et al., 2022; Shang et al., 2019). Genetic diversity is a fundamental prerequisite for species evolution and regulates the ability of populations to adapt to a range of factors such as e.g. global warming and is an important conservation target (Blows & Hoffmann, 2005; Hirsch et al., 2015).

Adonis vernalis L. forms a pontic-panonic sub-element, widespread in grasslands of the southeastern steppe zone of Europe and is characteristic for various xerothermic forests of continental-sub-Mediterranean type, belonging to the order *Festucetalia valesiaceae*. In Poland it is a relict species of the steppe zone flora (Denisow et al., 2008). *A. vernalis* is widely used in medicine in the treatment of cardiovascular diseases (Gostin, 2011; Shang et al., 2019 Zhumagul et al., 2024). As it is considered to be a threatened species, the species has been included in the IUCN Red List with the threatened status in Europe - Least Concern, although in some areas of Europe the threatened status may be assessed - extinction risk (Niculescu, 2023), it is also included in the synoptic red list for Central Europe, the species

is assessed as vulnerable by Schnittler and Gunther (1999).
The age characteristics of *A. vernalis* have been studied in different areas, resulting in the fact that the species has poor vegetative mobility, complexity of seed reproduction and low level of competition with other species in plant communities (Sultangazina et al., 2020).
Adonis vernalis is widely used in cardiovascular treatments due to its cardiotonic glycosides (Shang et al., 2019). However, overexploitation has led to a decline in wild populations, necessitating sustainable harvesting guidelines (Ghorbani et al., 2008).
The aim of this paper is to analyse the distribution and abundance of *Adonis vernalis* in 6 different natural habitats. The proposed objectives were designed to answer three different research questions: *i)* which are the limits of *A. vernalis* plant variability in their natural habitat?; *ii)* which are the potential limits of *A vernalis* in their natural habitats?; *iii)* at what level can be considered the natural habitats similar one to each other and how the similarity varies between habitats?

MATERIALS AND METHODS

The research areas were located on 3 grasslands around the city of Blaj (Alba County, Romania). Each of these regions were selected based on their potential to offer the ecological conditions for *A. vernalis*. The grassland with plots MF1, MF2 is located SW of the city, in a Natura 2000 habitat (Mănărade Grasslands - ROSAC0428 - Semi-natural dry grasslands and scrubland facies on calcareous substrates (*Festuco-Brometalia*). The grassland with plots OS1 and OS2 is located in the southern area of the city, and the one with samples OT1 and OT2 is in the same area, at a distance of 2 km. All grasslands areas are used through extensive grazing with cattle (in the period May-July), but without the application of any improvement management.
To accomplish the proposed objectives, 6 plots of 800 m^2 were selected in 2024 in the three targeted regions of study. Each plot was separated in 32 sub-plots, after an 8 x 4 scheme, that lead to a dimension of 25 m^2/sub-plot (2.70 m x 8.25 m). The selection of research plots was performed based on the presence of native *A. vernalis* in canopy and the presence of cattle for grazing. All the observation were performed in 2024, at the flowering stage of *A. vernalis*.
All data analysis was performed with the software Rstudio version 2024.12.0 (R Core Team, 2024), based on the multitude packages and statistical test. The packages used for basic statistics and histograms were "psych" (Revelle, 20024), "ggplot2" (Wickham, 2016) and "ggridges" (Wilke, 2024). Means, standard deviations and errors, medians, minimum and maximum of abundance were used to describe the abundance of *A. vernalis* in each of the six habitats. Histograms were created based on the abundance values observed in each habitat. The analysis of similarity (Anosim) was performed with „vegan" package (Oksanen et al., 2022), for the assessment of differences between and within the habitats in terms of plants abundance.

RESULTS AND DISCUSSIONS

The species *Adonis vernalis* is one of the species with conservation priorities at national and international level, it is a characteristic species for xerothermic grasslands of the continental-submediterranean type, belonging to the *Festucetalia valesiaceae* order (Zając, 2009). Different studies (Denisow et al., 2014) state that regarding the spread of *A. vernalis* populations, they have decreased considerably in several European countries, especially in recent decades. The main causes are overexploitation of populations (gathering for medical purposes) habitat disappearance (i.e. intensive agricultural practices) or overgrowing of patches due to secondary succession of grasslands (Forycka et al., 2004; Mihalik et al., 2000; Zając, 2009). Some causes of the disappearance of A. vernalis populations are also found in the biology and ecology of the plant, slow growth or problems with seed germination.
The six specific habitats of *A. vernalis* show a large difference in terms of basic statistics applied on plant number (Table 1). The means largely varied from 32 plants/plot up to a maximum of 113. Three of the analysed habitats (MF1, MF2 and OS1) showed means under 40 plants/plot, while two of the habitats showed average values of plants/plot of 63 (OT1) and more than 80 (OT2). Habitat OS2 is the only one where the mean of plants/plot reach 113 plants.

The calculated median of plants/plot in each habitat show a slightly different perspective than the calculate means. The difference observed compared to the mean for the habitats MF1, MF2 and OS1 is in the interval of 1-2 plants, with a similar situation for habitat OT2. On the opposite, for the habitat OS2 is visible an increase of median with 9 plants, while for OT1 was observed a decrease with 20 plants.

A. vernalis is a perennial plant that creates imposing tufts up to 50 cm high. The plants begin flowering in early April. *A. vernalis* is a species that reproduces generatively in natural conditions. The development of *A. vernalis* plants is slow and requires a particular composition of soil and proper weather conditions (Jankowska, 1988; Zhumagul et al., 2024a, 2024b).

The fluctuations in the comparison of means and medians are due to the minimum and maximum recorded in the field. The minimum values for MF1, OS1, OT1 and OT2 is 5 or bellow, while for OS2 is 22. These values are in contrast with the maximum recorded. Maxima values are visible in the habitat OS2 (240), to which only OT2 show close values (40 plants less). For the three habitats MF1, MF2 and OS1, the maximum values are near or below 100 plants/plot. Thus, the range obtained from ground counting is 218 for OS2, in the interval 160-200 for OT1 and OT2, and below 100 for the rest of three habitats.

The standard deviations calculated for the entire set of data indicate higher variability in each of the analyzed habitats (Table 1). For habitats MF1, OS1, OT1 and OT2, this parameter represents up to 60-80% of the recorded mean, while for OS2 less than 50%. The standard error is set in the range 5-10% for all the habitats, which enables the use of this parameter to calculate the maximum biological potential for each.

Table 1. Limits of *A. vernalis* target habitats based on the number of plants/25 m^2

Plot	Mean	SD	Median	Min.	Max.	Range	SE
MF1	36.69	25.49	35.00	3.00	100.00	97.00	4.51
MF2	33.09	13.13	31.50	10.00	73.00	63.00	2.32
OS1	31.56	21.55	32.00	1.00	97.00	96.00	3.81
OS2	112.97	56.69	121.50	22.00	240.00	218.00	10.02
OT1	62.59	52.69	49.00	1.00	165.00	164.00	9.31
OT2	82.75	60.58	80.00	5.00	197.00	192.00	10.71

Legend: SD – standard deviation; SE – standard error.

According to recent literature on *A. vernalis* populations, their persistence and development in habitats of high ecological value are closely linked to the dynamics of natural phytocenoses (Denisow et al., 2014; Lohvynenko et al., 2019). Factors influencing these dynamics, such as vegetation succession, natural disturbance regime and interactions between component species, play an essential role in ensuring optimal conditions for germination, growth and reproduction of *A. vernalis* (Denisow et al., 2014; Kulymbet et al., 2023). Thus, maintaining an ecological balance in these ecosystems is essential for the long-term conservation of populations of this valuable species (Gostin, 2011; Shang et al., 2019).

Although favourable habitats for *A. vernalis* are still widespread, populations of this species are subject to significant threats, mainly due to the abandonment of phytocenoses (Ghorbani et al., 2008; Łuszczyński and Łuszczyński, 2009). The lack of traditional interventions favours the excessive expansion of shrub species and other woody plants, which leads to profound changes in the structure of the vegetation. Dry grassland with *A. vernalis* has historically been extensively grazed with sheep and maintained by burning (Onoe, 2011; Nota et al., 2020). Fire events, in particular, act to increase *A.* vernalis population (Deák et al., 2014) when happens before flowering, while the occasional spring fires stimulates the germination of this species. One of the major effects of this process is the reduction of light at ground level, an essential factor for the germination and optimal development of *A. vernalis*. As habitats become increasingly shaded and competition for resources increases, populations of the species gradually decline, risking fragmentation and even local extinction (Sultangazina et al., 2020). Understanding its distribution and ecological requirements is essential for developing conservation strategies that maintain grassland biodiversity and its possible sustainable use for pharmaceutical purposes.

The frequency of *A. vernalis* distribution (Figure 1) in the plots form the analysed habitats show a large variation, due to the growth characteristics of this species. The trends of plant number distribution across all the six habitats (Figure 1) indicates that most of the analysed plots have a maximum of 50 plants/plot. The trend slowly is

decreasing in the interval 50-150 plants/plot, followed by a reduction near to zero observations. The pick value of plants is 40/plot and an abrupt decrease up to 75 plants/plot (first abrupt decrease), and a smooth decrease line up to the maximum of 240 plants/plot.

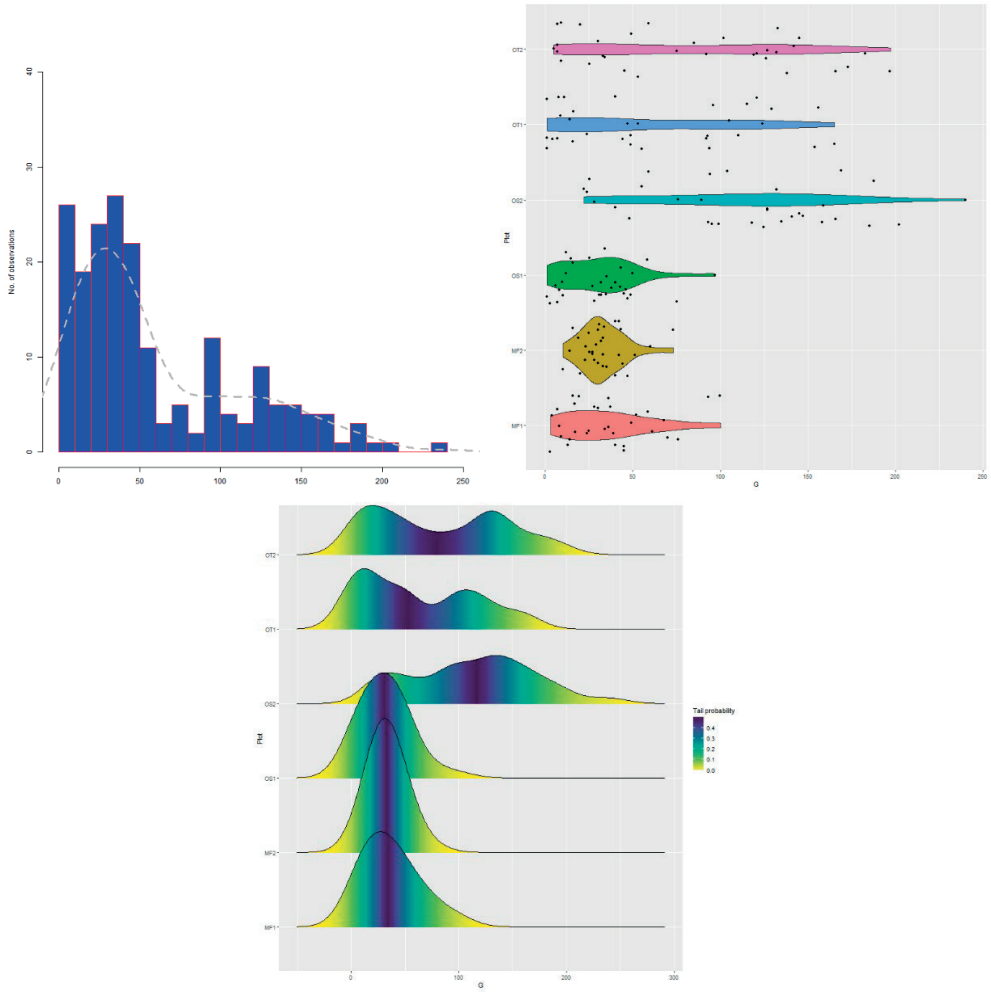


Figure 1. Distribution frequency of *A. vernalis* in natural habitats – presence vs. forecast

A case study conducted in the Akmola and North Kazakhstan regions highlights that the seed productivity of *A. vernalis* is significantly influenced by spring-specific climatic conditions, as well as by the stage of plant development (Sultangazina et al., 2020). Environmental factors, such as temperature, precipitation and the length of the growing season, play an essential role in the process of seed formation and maturation. Also, the age of the plants considerably influences the reproductive capacity, with more mature specimens generally having a higher seed production compared to young ones. Thus, annual variations in meteorological conditions, correlated with the biological cycles of the species, determine fluctuations in the reproductive success of the species in this region (Dmytrash-Vatseba, 2016; Sultangazina et al., 2020).

By analysing each of the habitat separately it can be observed a specific trend of plant distribution related to 25 m^2 plots (Figure 1). Three of the habitats (MF1, MF2 and OS1) show only one

probability of distribution, with a similar pick value of 30-40 plants per plot. This indicates a more homogenous distribution of plants in the field. The other three habitats show a two-tail probability curve, with one around 10 plants/plot and more than 120 plants/plot the second tail. The presence of two tails indicates a heterogenous distribution of plants in the field, with areas that present a large coverage of species and areas with sparse plants.

The number of plants at habitat level shows high fluctuations, with both heterogenous and homogenous distribution of plants (Figure 1C). For MF1, MF2 and OS1 the analysed plots show an equal distribution of observations across the entire interval of variation. For the rest of the three habitats, the distribution is across a longitudinal gradient, with a large variation between analysed plots in each habitat. These three habitats also show the high range between internal plots, with areas where plants are sparse and almost the same number of areas with a multitude of plants.

In the analysis of population dynamics of *A. vernalis*, one of the defining features is the length of the pregenerative period, which varies depending on the type of habitat. But why is this aspect so important? (Poshkurlat, 1969). First, if we look at cenopopulations in steppe areas, we observe that plants go through a much longer pregenerative period. This phenomenon can be explained by the more difficult environmental conditions: drier soil, reduced nutrient resources and large temperature variations impose on plants a survival strategy based on the slow accumulation of resources. Thus, young individuals spend several years in juvenile stages, before reaching maturity and contributing to reproduction (Denisow et al., 2014; Sultangazina et al., 2020).

In contrast, in grassland habitats, where the soil is wetter and richer in nutrients, plants no longer need a long period of vegetative development (Denisow and Wrzesień, 2006; Denisow et al., 2008). Here, the resources necessary for growth are more easily accessible, allowing for faster maturation and, implicitly, a reduction in the duration of the pregenerative stage. Consequently, populations in this environment have different dynamics, with a higher replacement rate of mature individuals. This variation in the duration of the pregenerative period directly influences the age structure of the population, an essential factor in determining its sustainability. A balanced population must contain a sufficient number of individuals at all stages of development: seedlings, juveniles, adults and senescent plants. If the age structure is unbalanced – for example, if there are too few young individuals capable of replacing mature plants – the population may be in danger of decline. This aspect is also crucial from the perspective of the population's self-sustaining capacity. In steppes, where plants regenerate more slowly, any external intervention – such as excessive grazing or harvesting for medicinal use – can have devastating long-term effects. In grasslands the regeneration capacity is higher, allowing the population to maintain its balance more easily. Therefore, the study of age dynamics and the pregenerative period in *A. vernalis* is not just a theoretical aspect, but one with concrete implications for the conservation of the species. Understanding these mechanisms can help develop effective strategies to protect habitats and maintain stable populations over the long term.

Distribution in habitat, along with cover in plant community, is a biological characteristic of *A. vernalis*. Thus, between the 6 selected habitats is visible a large dissimilarity, determined by the number of plants present in each habitat and their allocation in sampling plots (Figure 2, Table 2). The ANOSIM analysis presents a significant difference between habitats, which is important for the detection of multiple distribution characteristics of this species.

There is a gradual, non-linear, increase in habitat dissimilarity along with the increase in the number of plots compared (Figure 2, Table 2). The lowest values of this indicator are visible when a low number of plots are compared. As the number of plots compared increase, it is visible that the increase of dissimilarity. For the entire set of plots, the dissimilarity shows high values, which is representative for a high heterogeneity of plant distribution across all the habitats. On the other hand, when the dissimilarity is compared within habitats, there are three different phenomena.

The first one is related to MF1 and MF2 habitats, where the plants are distributed heterogenous across the entire sampling area (Figure 2, Table 2). This produces a heterogenous pattern, with

the alternance of areas that present a medium number of plants with areas that present sparse plants.

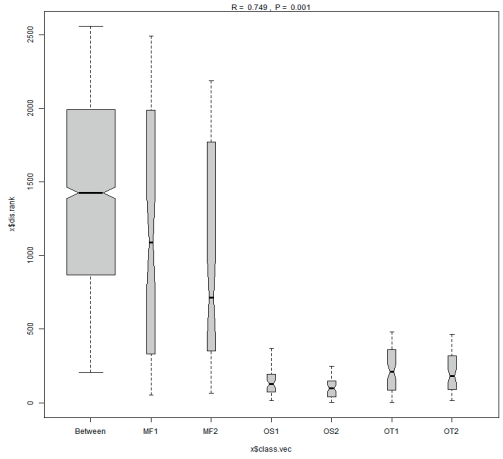


Figure 2. Similarity and disimilarity of *A. vernalis* natural habitats

Table 2. Limits of *A. vernalis* target habitats based on the number of plants/25 m^2

	0%	25%	50%	75%	100%	N
Between	206	867.75	1425.5	1990.25	2556	2160
MF1	52	343	1089.5	1968.25	2489.5	66
MF2	67	359.5	716	1747.5	2187	66
OS1	16	73.75	127.5	192.5	368	66
OS2	5	42.5	97	145.5	250	66
OT1	1	88.25	212.5	357.5	483	66
OT2	14	90	181.5	316.25	466	66

Note: ANOSIM statistic R: 0.7488 / Significance: *0.001*.

The second phenomenon is visible for habitats OT1 and OT2, that present low to medium number of plants within the habitat, but their pattern of distribution is more homogenous within the habitat (Figure 2, Table 2).

The third phenomenon is composed by two distinct situations (Figure 2, Table 2). Habitat OS1, that presents medium to low number of plants, but with a homogenous distribution across the habitat. And OS2, where the number of plants is in the range medium-high, but their distribution is homogenous across the habitat, making the cross from one plot to another one smoother.

Given the medicinal importance of the species, we recommend further research on domestication potential and agro-ecological cultivation techniques. The sustainable harvesting of Adonis vernalis is crucial to prevent population decline, as the species has a slow regeneration rate and is highly sensitive to habitat disturbance (Denisow et al., 2014; Sultangazina et al., 2020). Studies indicate that overharvesting, primarily for medicinal purposes, has led to significant reductions in population densities across Europe, particularly in fragmented grassland ecosystems (Forycka et al., 2004; Mihalik et al., 2000). To ensure long-term conservation, experts recommend harvesting only mature individuals after seed dispersal, limiting annual collection to no more than 30% of a local population (Ghorbani et al., 2008; Shang et al., 2019). Additionally, habitat management strategies such as rotational harvesting, controlled grazing, and protection of key populations in Natura 2000 sites are essential for maintaining ecological stability (Łuszczyński & Łuszczyńska, 2009; Niculescu et al., 2023). Future research should focus on the potential for cultivating *A. vernalis* in controlled environments as an alternative to wild collection, reducing pressure on natural populations while maintaining medicinal resource availability (Gostin, 2011; Hirsch et al., 2015).

Economic valorisation of grasslands through the sustainable use of medicinal species, such as *Arnica montana*, has been shown to significantly increase landowners' interest in maintaining these habitats, thereby contributing to biodiversity conservation (Păcurar et al., 2023). Studies indicate that when traditional hay meadows and oligotrophic grasslands are managed for high-value products, local stakeholders become more engaged in habitat preservation, ensuring the long-term ecological sustainability of these ecosystems.

Integrating advanced monitoring techniques, such as those presented in the study "Detection and Quantification of *Arnica montana* L. Inflorescences in Grassland Ecosystems Using Convolutional Neural Networks and Drone-Based Remote Sensing" by Sângeorzan et al. (2024), can significantly enhance the accuracy and efficiency of population assessments for medicinal plant species like *Adonis vernalis*. By employing unmanned aerial vehicles (UAVs) equipped with RGB sensors and advanced image analysis algorithms, researchers can effectively detect and quantify *A. montana* inflorescences, facilitating more precise monitoring of their habitats. Applying similar methodologies to *A. vernalis* could improve

conservation efforts by providing detailed spatial data on population distributions, aiding in the development of sustainable harvesting practices and habitat management strategies (Sângeorzan et al., 2024).

CONCLUSIONS

Our findings indicate significant habitat variability in *A. vernalis* populations, with implications for both conservation and sustainable use.

The *A. vernalis* natural habitats show large heterogeneity phenomena, with a fluctuating abundance of plants even at small scale analysis. The presence and abundance of *A. vernalis* species varies greatly between different natural habitats, with an average of 50 plants/25 m^2, and a site-specific trend of abundance. Based on field observations the highest number of *A. vernalis* plants/25 m^2 can reach 240 individuals, which is considered a very abundant habitat type.

The natural abundance of *A. vernalis* shows two presence scenarios. The first one represents a homogeneous distribution of plants, with a pick value of 30-40 plants/25 m^2. The second scenario indicate a heterogeneous distribution, with two specific cases – 10 plants/25 m^2, respectively 120 plants/25 m^2.

High-density populations such as those in OS2 could support controlled harvesting, while low-density habitats like MF1 require conservation priority and habitat restoration.

Conservation strategies should focus on maintaining open grassland ecosystems through sustainable land management practices. Long-term monitoring is essential to evaluate the impact of ecological changes and inform adaptive conservation measures.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

INFLUENCE OF NITROGEN FERTILIZATION ON TWO-ROWED WINTER BARLEY GENOTYPES UNDER 2023-2024 YEAR IN SOUTHEAST OF ROMANIA

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Abstract

This paper presents a one-year study (2023-2024) of 10 winter 2-rowed barley genotypes concerning the effectiveness of nitrogen fertilization on quality parameters that seeds need to accomplish for malting barley. The experiment was conducted at National Agricultural Research and Development Institute Fundulea, in Southeast Romania. The genotypes were sowed in the 2023 year in two technological sequences in 3 replications (T0 - 0 kg urea/ha and T1 - 100 kg urea/ha). It was revealed that were no significant differences in yield between the unfertilized (T0) and fertilized (T1) experience, the difference between the mean value of the experience was 635 kg/ha. The lowest value for protein content to be accepted by the malting industry was registered by the Gabriela variety (T0 - 11.47%) meanwhile the highest value of the experiment was registered by the Ileana variety (T1 - 15%) which indicates a suitable variety for animal forage. Starch content ranged between 61.2% (T0) and 63.9% (T1). A significant difference was observed in grain plumpness between the technological sequences as follows: the mean of the dataset for (T1) was 94.04% compared with 69.51% (T0).

Key words*: barley, variety, quality indicest.*

INTRODUCTION

Barley (*Hordeum vulgare* L.) is one first domesticated small-grained cereal crops globally (Badr, 2000). In Romania, barley is cultivated on 497,770 ha^{-1} with a total production of 1,997,620 tonnes/ha^{-1}, ranking 4th for crop production after wheat, maize, and sunflower (FAOSTAT, 2023). The farmers prefer winter barley varieties with 6 rows because of their higher yield, more stress-tolerant to drought, and more tolerant to winter conditions (Samarah et al., 2009). In contrast, 2-row varieties (mostly for malting) offer higher grain quality, are more resistant to lodging, and have stronger straw (Narasimhalu et al., 1988).

Water deficit causes undesirable effects on grain yield by decreasing photosynthesis activity and accelerating leaves aging (Gerik et al., 1996), limiting cell growth, leaf expansion, and transpiration (Hsiao, 1973).

Thousands grain weight (TGW) expressed in grams is one the most important traits of the obtained yield (Hadjichristodoulou, 1990). The grain filling period is influenced by the environment and it was reported to be low correlate with weight and seed size (Coventry et al., 2003). Seeds with high values of TGW assure an increased content of starch and a low protein content (Burger and La Berge, 1985).

High protein content in the seeds affects the malt extract, including efficiency, time of extract, and profitability (Paynter, 1996), and is influenced by the nitrogen fertilization related to the growing stage, applied rates, and natural environmental nitrogen existing in the soil (Chen et al., 2006).

Barley breeding programs focus on developing genotypes thatpossess increased yield potential, high adaptability, and high responses to agronomic inputs (Pržulj et al., 2014). Grain protein content in barley is highly associated with feed quality and malt quality. A high value for protein content is favorable for feed and forage while a low protein content is suitable for malt quality (Shengguan, 2014). Grain protein content affects malting quality, the most important being yeast nutrition, beer turbidity, and enzyme activities (See et al., 2002; Clancy et al., 2003). Grain protein content is influenced

by the genotype and environment (Jukanti et Fischer, 2008; Smith, 1990). In dry conditions, nitrogen translocation is necessary for cereal production. The heat stress after the flowering phase induces drought stress which limits the nitrogen translocation (Kirda et al., 2001) so yield and protein content are dependent by assimilates translocated from vegetative tissues before flowering (Pheloung and Siddiqquea, 1991). The main objective of the present paper is to asses the influence on nitrogen fertilization on yield and quality parameters of malting barley in 2023-2024 in Southeast of Romania.

MATERIALS AND METHODS

Researches were performed in the 2023-2024 agronomical year in the barley breeding field, on a cambic chernozem in Southeast Romania, at the National Agricultural Research and Development Institute Fundulea (Latitude 44.4547°N, Longitudine**:** 26.5155° E). The analysed genotypes were 10 winter 2-rowed barley (5 varieties and 5 perspective lines), all created at NARDI Fundulea. The sequence of testing genotypes was nitrogen fertilization in 2 rates (T0 - 0 kg of Urea/ha^{-1} and T1 - 100 kg urea/ha^{-1}) applied on the 15th of March, 2024. The soil was tilled in autumn 2023 by harrowing followed by cultivation. The sowing was performed on the 20th of October, 2023 in randomized plots in 3 replications of 6 m^2, at 12.5 cm between rows on a density of 350 seeds/square meter. The weed control has occurred using Stomp Aqua (455 g/l Pendimetalin) applied at a dose of 3 liter/ha^{-1}. In spring it was necessary to spray a second time for weed control, using Axial One (45 g/l pinoxaden, 5 g/l florasulam, 11.25 g/l cloquintocet-mexil) with a dose of 0.8 l/ha^{-1}. The plots were harvested on 20th June 2024 with Wintersteiger Delta combine for the experimental plot. The yield was weighted for 4.5 square meters at 14% moisture, then reported for ha^{-1}. After the yield was assessed, samples were prepared in 3 replications and reached the quality laboratory for further analyses:- thousand grain weight was assessed using a Contador instrument (set by 1000 grains) followed by weighing on a electronic balance.

- assortment (seeds bigger than 2.5 mm diameter) as percent from 100 grams of sample, determined with Sortimat (2.5 mm sieve)
- protein and starch content were analyzed by INFRATEC 1245 (%)

The datasets were analyzed with SPSS version 26. ANOVA with 2 factors was determined to see if the analysed dependent variables were statistically influenced by chosen factors (genotype, fertilization, and the interaction between them). In addition, descriptive statistics were determined (mean, maximum, minimum value) for each category of data.

Total rainfall registered in 2023-2024 (Table 1) from sowing to harvesting was 308.8 mm, with -106.2 mm less than the multiannual average of 415 mm, registered at NARDI meteorological station (mean of 60 year).

The temperatures (Table 2) were higher than the multiannual average with almost 4°C for the entire growing season, the maximum difference occurred in February when the temperatures were at the multiannual average of 8°C.

Table 1. Rainfall (mm) at NARDI Fundulea in the 2023-2024 year

	Month									
	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun	Sum
Rainfall	29.0	85.6	24.4	17.6	1.4	38.6	62.4	34.2	15.6	308.8
Average	42.3	42.0	43.7	35.1	32.0	37.4	45.1	62.5	74.9	415.0
Dif	-13.30	43.60	-19.30	-17.50	-30.60	1.20	17.30	-28.30	-59.30	**-106.20**

Table 2. Temperatures at NARDI Fundulea in the 2023-2024 year

	Month									
	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun	Mean
T(°C)	16.1	8.5	4.3	1.0	7.6	8.5	15.0	16.4	26.1	11.5
Average	11.3	5.4	.0	-2.4	-.4	4.9	11.3	17.0	20.8	7.5
Dif	4.80	3.10	4.30	3.40	8.00	3.60	3.70	-.60	5.30	**3.96**

*multiannual average (60 years)
**difference between registered rainfall during the experimental year and multiannual average

RESULTS AND DISCUSSIONS

Grain yield

The yield was significantly influenced by the genotype and fertilizer rate but not significantly influenced by their interaction (Table 3)

Thousands grain weight

Regarding TGW (g) the obtained results registered a difference of 0.75 grams between the average of the analyzed categories (Figure 3).

Table 3. Two-way ANOVA for studied parameters

Source	Yield (kg/ha^{-1})	TGW (g)	Assortment (%)	Protein content (%)	Starch content (%)
Genotype (G)	.000*	.000*	.000*	.000*	.000*
Fertilization (F)	.000*	.000*	.000*	.371^{ns}	.352^{ns}
G x F	.048^{ns}	.000*	.000*	.086^{ns}	.061^{ns}

*The mean difference is significant at the .05 level.

The difference between the yield average of different nitrogen rates was 635 kg/ha^{-1} (Figure 1). Comparing the average yield between the genotypes can be observed that the highest difference between the two nitrogen rates was obtained for DH 417-12 (+1195 kg/ha^{-1}) and DH 403-12 (+978 kg/ha) for T1, while Gabriela variety reached a difference of 683 kg/ha^{-1} for T0 (Figure 1).

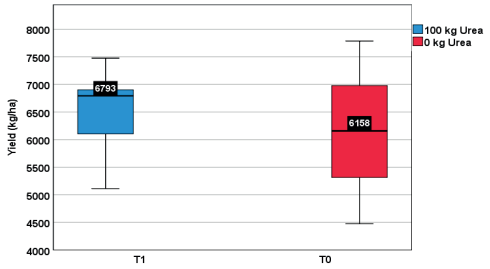


Figure 1. Average yield between two fertilization rates

The most stable genotypes with the lowest variations were Artemis, DH 425-3, DH 425-4, Diana, and the used Check.

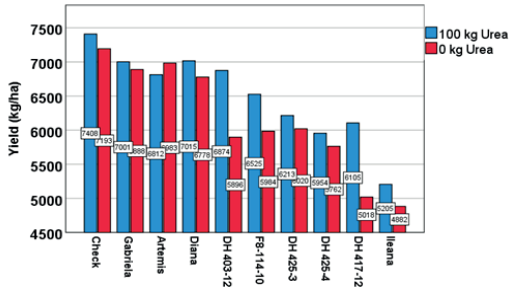


Figure 2. Average yield between two fertilization rates registered for experimented genotypes

The thousands grain weight registered the highest values for the T1 rate for 7 genotypes except only 3 of them which had better results for the T0 application. The biggest difference that this parameter was registered for the Gabriela variety (+3.0 g) and used Check (3.3 g). Ileana variety registered the lowest values both for T1 and T0, below 44.0 g (Figure 4).

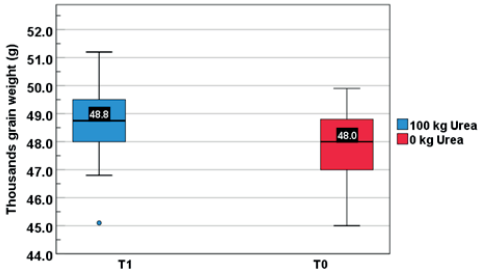


Figure 3. Average TGW (g) between two fertilization rates

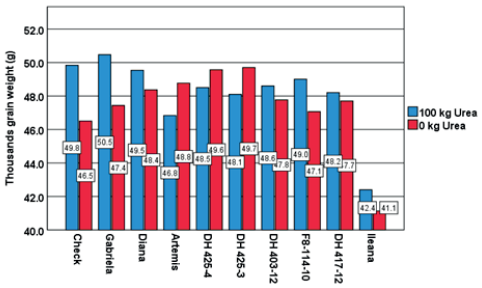


Figure 4. Average TGW(g) between two fertilization rates registered for experimented genotypes

Assortment

Nitrogen fertilization had a very significant impact on seeds size as it is shown in Figure 5.

The difference between TGW(g) for compared categories was 23.7%.

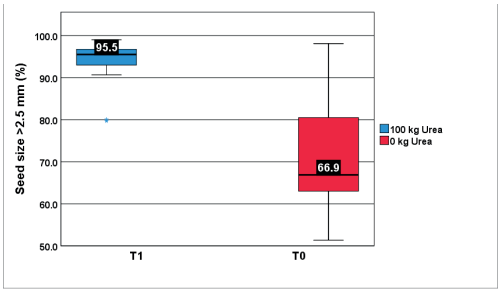


Figure 5. Average seed size (g) between two fertilization rates

Comparing each genotype, the registered values had better responses for the application of nitrogen (T1). Using this sequence the plumpness of seeds is higher than 90% (seeds bigger than 2.5 mm diameter) for almost all tested genotypes except the Ileana variety (only 80%). The highest seed size was registered for DH 417-12 (98.24%) T1, while T0 had very negative effects for the Ileana variety (51.67%) seeds bigger than 2,5 mm diameter (Figure 6). The most stable variety for this parameter was Artemis with a difference of 6.3% between the two fertilization rates.

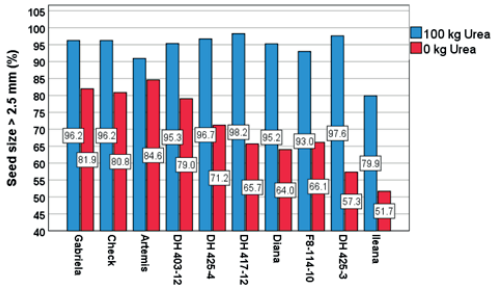


Figure 6. Average seed size (g) between two fertilization rates

Protein content

During studied year it was shown in Table 3. That the protein content was significantly influenced by the genotype but not by the nitrogen fertilization. It is a slightly decrease between the two categories but with no significant impact on the protein content. Regarding the response of the genotype, the protein content has been significantly influenced. The average value for the two categories registered a difference of 0.5% (Figure 7).

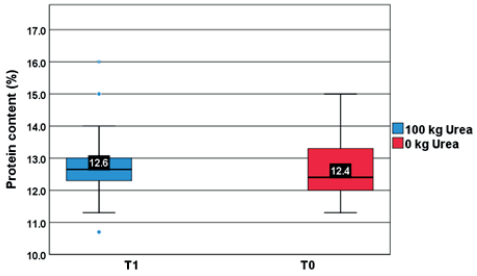


Figure 7. Average protein content (%) between two fertilization rates

The protein content ranged from 11.47% (Gabriela - T0) to 15.00% (Ileana variety T1). Ileana variety registered high values for both fertilization rates (exceeded 15% for T1).

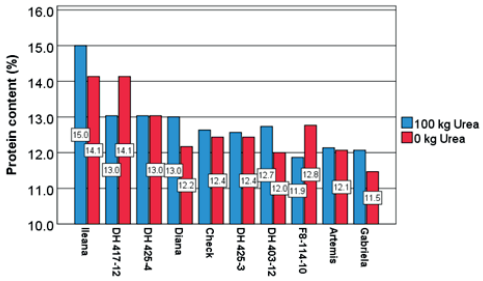


Figure 8. Average protein content between two fertilization rates

The highest difference between two fertilization rates was registered at DH 417-12 (1.56%). The most stable varieties which registered the lowest difference between the two fertilizations strategies were Artemis, DH 425-3, DH 425-4, Gabriela, and the check, which maintain the protein values almost the same.

Starch content

The starch content registered in the experimented year had a slight increase from a minimum of 62.17% (T1) to 62.28% (T0), an opposite effect compared with the other parameters, but statistically insignificant.

The studied 2-rowed genotypes for registered starch content had a better response for N0 fertilization, (Figure 10) shows that the graphic line for T0 starch content is above the T1 line except for DH 417-12, DH 425-4, and the check.

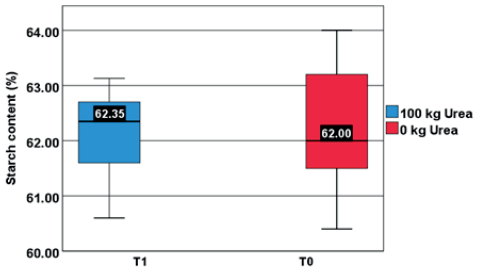


Figure 9. Average starch content (%) between two fertilization rates

The highest value was registered at the Ileana variety for both conditions (63.0% - T0 and 63.0% - T1). On a background of T0, Artemis (+0.5%) and DH 403-12 (+0.63%) variety had a better response regarding starch content.

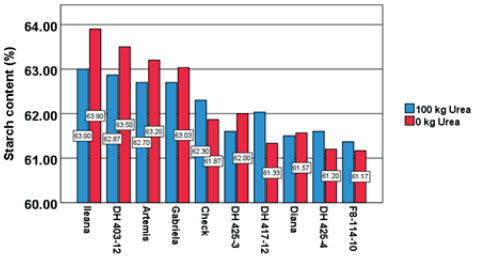


Figure 10. Average protein content between two fertilization rates

CONCLUSIONS

The results obtained during the 2023-2024 year highlighted that the highest values for yield, thousand grain weight, seed size, and protein content were realized using 100 kg of urea (T1). The starch content had the opposite effect, but insignificant, being higher for T0 (without fertilizer application).

The highest yield registered for the Gabriela variety (7684 kg/ha^{-1}) reveals the high adaptability of this variety to drought conditions which have been more frequent in the last year in the southeast of Romania. During the experimental year, we can conclude that in this drought year 2023–2024, nitrogen fertilization was not beneficial for all varieties, some of them being unable to use it due to a lack of optimal growing conditions (Tables 1 and 2).

The thousands grain weight was also affected by the heat and water stress. It was shown that despite those conditions, the Gabriela variety succeeded in thousand grains weight over 50.0 g which empowers this trait as being suitable for the malting industry. The importance of the fertilization strategy was also revealed for the check values which had better response for 100 kg fertilizer.

The effect of seed size was the most affected by the 2023-2024 conditions. Seed size registered a low percent for T0 for all studied genotypes, underlying that without nitrogen the seed size could be affected for malt extract acceptance. The Artemis, Gabriela, DH 403-12 and standard chech registered over 75% of seeds bigger than 2.5 mm in diameter which can be selected both for breeding and selecting for the malting industry.

Protein content was significantly different ($p < 0.05$) only between genotype responses. On a background of nitrogen fertilization, the protein content during the experimental year ranged between 12 -13 % for almost all studied genotypes. Gabriela variety and F8-114-10 line had a low protein content which indicates that raw material is suitable for the malting process. The Ileana variety registered the highest values of proteins for T1 and T0 respectively (over 14%), highlighting this variety as a good row material for animal forage.

Starch content response was the most different compared to the other parameters. The starch accumulated in seeds tends to increase using no fertilizer (T0). Again, the Ileana variety had a good response in this trait for animal forage (starch content exceeded 63% for both conditions).

In conclusion, in the 2023-2024 year, drought background the 2-rowed winter varieties created at NARDI Fundulea had suitable results for malting and animal forage too. The yield, TGW, seed size, low protein content, and starch content for Artemis and Gabriela for both conditions (with and without fertilizer) recommend those varieties suitable for malting, while the Ileana variety is more valuable for animals (high protein and starch content).

Further research would be needed to establish that analyzed parameters tend to maintain or change the tendency registered in the studied year.

ACKNOWLEDGMENTS

This research paper was caried out with fundings provided by the Ministry of Agriculture and Rural Development Romania, for sectorial project ADER 1.1.4/23.07.2023.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

EFFECT OF METEOROLOGICAL CONDITIONS ON THE PRODUCTIVITY OF MAIZE HYBRIDS OF DIFFERENT MATURITY GROUPS

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Abstract

The work aimed to study the productivity of maize hybrids from maturity groups FAO 300 to 600 under different conditions. A four-year experiment was carried out at Maize Research Institute Knezha, Bulgaria. The hybrids Kneja 310, Kneja 461, Kneja 565 and Kneja 683 A were grown under non-irrigation conditions and with approval for the regional agricultural technology. Two-factor analysis of variance was used to find the effect of both hybrid and environment on the grain yield. In addition, Stability Yield Index (SYI) was calculated. The amount of rainfall per growing season varied from 199.8 to 316.1 mm/m^2, and grain yield averaged for the tested hybrids by years varied from 859.7 to 1040.7 kg/da. The highest average grain yield over the period was obtained from Kneja 565 (1003.9 kg/da) and the lowest - from Kneja 310 (900.3 kg/da), respectively. Kneja 683 A hybrid showed the low Stability Yield Indices, 0.685 followed by Kneja 565 0.729 values, respectively. Relatively high and stable yields were obtained from Kneja 310 (900.3 kg/da) and Kneja 461 (925.9 kg/da) with SYI 0.937 and 0.841, respectively.

Key words*: grain yield, maize, Stability Yield Index.*

INTRODUCTION

The climate changes we are all witnessing are affecting maize productivity (Christensen et al., 2013; Elbehri et al., 2015). Some model projections identify grain maize as one of the crops most vulnerable to climate change in Europe (https://www.actualno.com/climate/klimatichni-promeni-kak-shte-povlijajat-na-uslovijata-za-otglejdane-na-carevica-u-nas-news_2073706.html). The growing season of maize is located in spring and summer when the daylight hours are longest, the maximum temperatures are highest, and rainfall is unevenly distributed with an annual maximum in May and June. Prolonged droughts are common occurrence in July, August and September. The crop's productivity is very dependent on temperature and rainfall during the growing season and especially during the critical stages of its development. Later in its development, it is less demanding of moisture, but highly sensitive in the silking and flowering stages, when it uses up nearly 50% of the water required for the entire growing season. Lack of moisture during flowering has a negative effect on pollination, and the water deficit during grain formation and filling affects grain size.

Current research and models are bringing to the fore resistant and adaptable varieties and hybrids, as well as the right choice of vegetation group of maturity suitable for each region (Cairns et al., 2013). The preference is for hybrids with early flowering (before the intense heat) and short growing season, also suitable as second crops, with a vigorous start, resistant to stress and disease, and diversity in the crop for better pollination. During flowering, the optimum temperature is 25-28°C, values of 30-35°C, combined with low atmospheric humidity, adversely affect pollen viability.

Productivity is important, but also yield stability. High and stable yield is a very important characteristic of maize cultivars. A stable cultivar yield means that its rank relative to other cultivars remains unchanged in a given set of one environment during several years. Some results revealed that high-yielding cultivars also can be stable cultivars (Abera et al., 2006).

Our study aimed to establish the performance of maize hybrids of FAO 300 to FAO 600 maturity group under different agrometeorological conditions.

MATERIALS AND METHODS

The experiment was carried out at the experimental field of Maize Research Institute - Knezha, Bulgaria under non-irrigated conditions during the period from 2020 to 2023. Four hybrids from FAO 300 to FAO 600 maturity groups were tested as follows: Kneja 310, Kneja 461, Kneja 565 and Kneja 683A, all of them part of the breeding collection of the Institute. A block method was used, 4 replications, the size of the experimental plot was 10 m^2 and the density of 5300 plants/da. The soil type was typical chernozem. The standard technology of growing of maize was applied.

Meteorological conditions, viz. rainfall and daily average temperature of growing seasons, were recorded. In addition, the meteorological conditions for a long-term period (1991-2020) were shown. Hydrothermal Coefficient (HTC) was calculated for the reproductive stage (sum of precipitation and accumulated sum of daily temperature from last decade of June and all days of July) for each year of study. To calculate the Hydrothermal coefficient the next formula, as suggested Selyaninov, was applied: HTC= Σ x / Σ t x 10; where Σ x and Σ t – accordingly sum of precipitations and temperatures in the period. The interpretation of the hydrothermal coefficient according to Selyaninov was based on the following: HTC from 1.0 till 2.0 – humidity is sufficient; HTC > 2.0 – immoderately humid; HTC < 1.0 – insufficient humidity; HTC from 1.0 till 0.7 – dry; HTC from 0.7 till 0.4 – very dry.

The grain yield was calculated in kg/da and it was reported at moisture content of 14%. Factorial regression was performed to obtain a biological explanation of the hybrid, environment, and hybrid - environment interaction for the yield. Stability yield index (SYI) was applied to determine hybrid yield stability. The formulae as suggested Singh et al. (1990) was used: SYI (Sustainable yield index) = (Ym - Sd)/Ymax, where Ym – mean yield; Sd – standard deviation; Ymax – the maximum yield. Statistical analysis of the experimental data using ANOVA without and with replication processing was performed using the SPSS 20.0 software product for Windows.

RESULTS AND DISCUSSIONS

The agrometeorological conditions during the growing season are a very important factor significantly affecting the growth, development and productivity of maize. The meteorological conditions comprising the growing season of maize over a long-term period (30 years) are presented in Figure 1. From the data, it can be seen that the mean daily temperature increases gradually from April to July and August. The most significant temperature difference was found between April and May (4.1°C), while between May, June and July it was about 3°C. The highest monthly mean daily temperatures were recorded in the typically summer months of July and August, with values very similar.

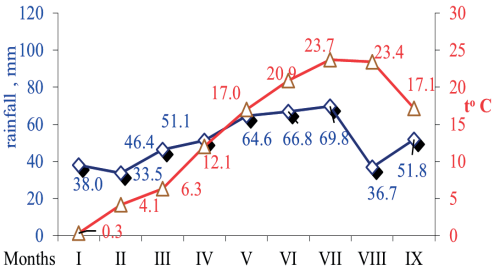


Figure 1. Meteorological conditions including the growing seasons of maize for the long term 30 years period 1991-2020

Monthly rainfall totals increase from the beginning of the growing season in April to July, again with the most significant difference between April and May (about 14 mm), while between May, June and July (65, 67 and 70 mm), the difference ranged from 2 to 5 mm. A significant decrease in the rainfall total for this long-term average period was recorded in August (37 mm), with a total for the growing season of 289.0 mm.

For the 2020-2023 study period, the meteorological conditions during the maize growing season by year are presented in Table 1. A gradual increase in mean daily temperature occurs from April to July and August. In May and June the temperature was above 16°C and above 20°C, respectively, with higher values of 17.4°C and 21.6°C only in 2022.

Comparing the differences of the monthly mean daily temperatures of April and May over the long-term period with those of the study years, the differences were found to increase from 4.1°C to 5.0°C, 6.0°C, 6.2°C and 5.0°C for 2020, 2021, 2022 and 2023, respectively. This accelerated warming at the beginning of the growing season in four consecutive years is indicative of a change in the environmental conditions during the vegetative stage of crop development. In addition, July 2023 was 0.25°C above the record - since measurements began in the world at large.

The temperatures were found highest in July, with only 2020 recording a higher value for August (23.5°C). The average temperature for the growing season was about 19.0, with only that in 2022 close to 20°C. The highest average daily temperature values were in July (24.1°C) and in August (23.9°C). Maitaha et al. (2021) found that yield was negatively correlated with temperatures in July and August. The high temperatures in July coincide with the reproductive stage of maize development, which can have an extremely negative effect on the yield as also suggested by Shiduo et al. (2024).

In terms of precipitation, over the four-year study period, precipitation amounts ranged from 199.8 to 316.1 mm for 2020 and 2021, respectively (Table 1).

Table 1. Agrometeorological conditions during the growing seasons of maize for the period 2020-2023

Months/ Years	2020	2020	2021	2021	2022	2022	2023	2023
	mm	to °C	mm	to °C	mm	to °C	mm	to °C
IV	8.9	10.7	46.9	9.15	67.3	11.0	45.6	10.9
V	38.7	16.7	82.0	16.9	35.1	17.4	71.5	16.1
VI	75.7	20.7	95.4	20.6	75.1	21.6	92.6	20.4
VII	49.4	22.9	35.2	24.3	7.5	24.7	71.3	24.6
VIII	27.1	23.5	56.9	23.9	74.8	24.2	23.8	24.2
Sum/av	199.8	18.9	316.4	19.0	259.8	19.8	304.8	19.2
CV%	62.5	28.1	39.3	32.9	57.4	28.8	43.7	30.1

The month with the highest monthly rainfall amount was June and has the least variation during the period 2020-2023, while the month with the lowest amount was July and has the highest variation. On average over the period, the highest rainfall amount was recorded in the months of May and June, while in the remaining months the rainfall amount values were similar (above 40 mm).

It is evident the precipitation was very irregularly distributed by months. The total precipitation amounts for the growing season fluctuated by year. The coefficient of variation (CV%) for precipitation ranged from 39.3 to 62.5% and was significantly higher than that for mean daily temperature, which ranged from 28.1 to 32.9%. The smallest amount of precipitations was recorded in 2020, being 26% less than the average for the period. The highest precipitations as a sum were recorded during 2021 and 2023, exceeding the average for the period by 17 and 13%, respectively.

Undoubtedly, precipitation is the most important factor influencing the maize grain yield (Huang et al., 2015). How this precipitation both by month and as total sum, influenced the maize growing and development and also the yield of grain is not so clear. To clarify the importance of precipitation, we calculate the hydrothermal coefficient as suggested by Selyaninov (1928) for the main stages of the reproductive period of maize (Figure 2). For the location of Knezha this period begins relatively from June 20th and continues throughout the whole month of July. The calculation of the ratio of the average daily temperature sum and the precipitation through HTC characterizes in more detail the conditions in which this important reproductive stage of development of maize occurs (Qiao et al., 2025).

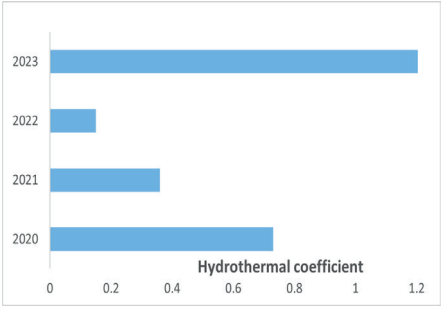


Figure 2. Hydrothermal coefficient of Selyaninov for the vegetation period of maize

It is evident from the HTC calculated values by year that they do not correspond to the rainfall amounts during the growing season. For example, the 2020 growing season has the least amount of rainfall (199.8 mm), but this during the reproductive stage together with the temperature amount formed a coefficient of 0.73, while the 2021 growing season has the highest amount of rainfall (316.4 mm), the HTC coefficient was twice as low (0.36). The lowest

hydrothermal coefficient for the reproductive stage was recorded in 2022 (0.15), despite the close precipitation amount to the average for the growing season (259.8 mm). This is mainly due to the scanty rainfall in July of 2022. The highest value of HTC was recorded in 2023 due to significant rainfall during the last ten days of June and the whole month of July.

Weather conditions affect the formation of grain yield of maize. The reported grain yields of maize hybrids are presented in Table 2. Many authors confirm the influence of weather conditions on maize grain productivity (Wei et al., 2021; Rajičić et al., 2024). In the last 25 years, grain yield has become increasingly dependent on weather conditions during the maize growing season, which have often involved "extreme climate events" (Bekavac et al., 2010).

Table 2. Grain yield from maize hybrids (kg/da)

Hybrid/ Year	2020	2021	2022	2023	Average	SYI
Kn 310	831.3	911.1	893.1	965.8	900.3	0.937
Kn 461	911.4	901.6	898.5	992.4	925.9	0.841
Kn 565	894.4	1058.7	893.4	1168.9	1003.9	0.729
Kn 683A	905.1	802.3	753.9	1035.8	874.3	0.685
Average	885.6	918.4	859.7	1040.7	926.1	
CV%	4.16	11.51	8.21	8.67	6.04	
LSD 0.05	38.9	110.5	114.3	138.9	68.9	

Grain yields in the first experimental year varied within a relatively narrow range (from 831.3 to 911.4 kg/da for Kneja 310 and Kneja 461, respectively). The HTC value was 0.73, which defines the conditions of the year at the generative stage of maize development as dry. In such a year, our data showed that the variation of grain yields was low (CV% 4.16). Under very dry conditions in this period, such as those of the experimental year 2022 (HTC 0.13), yield variation was also low (CV% 8.21) and all hybrids tested (except for Kneja 683A) had similar yields. In 2021 at HTC 0.36, yield variation was average in strength (CV% 11.51), with the highest yield recorded in Kneja 565 (1058.7 kg/da). In the fourth year, which was meteorologically the most favourable, the highest yields were also recorded. For the Kneja 565 hybrid the yield reached 1168.9 kg/da.

On average for all hybrids, the highest yield was obtained in 2023 (1040.7 kg/da), which is 12.4% more than the average yield obtained over the entire four-year experimental period. This was followed by the yield in 2021 (918.4 kg/da), with the lowest yields in 2022 and 2020. Then the amount of grain obtained is 859.7 and 885.6 kg/da, or 7.2 and 4.4% lower than the average for the period in the absence of statistical evidence.

Comparing the average yields of the tested hybrids by year with the recorded rainfall during the maize growing season (April-August), there is some correspondence of the amount of rainfall with the average yields obtained. For example, the highest average yields in 2023 and 2021 were consistent with the highest amounts of rain recorded during the growing seasons, 304.8 and 316.4 mm, respectively. The rainfall totals of 259.8 and 199.8 mm in 2022 and 2020 produced the lowest average yields.

The highest average yields in 2021 and 2023 were with a difference of more than 120 kg. In 2023, rainfall amounts are very similar.

Looking at the rainfall amounts by month of the growing season, it is clear that the amounts in April, May and June are very close. However, the differences in July and August are significant. In July of 2021, the rainfall is twice that of 2023 (35.2 and 71.3 mm), while in August it is the opposite - twice the amount in 2021 (56.9 mm) compared to 23.8 mm in 2023. In April of 2020, rainfall was only 8.9 mm, while that in 2022 was 67.3 mm. In May and June the amounts were similar, but in July the amount in 2020 was many times greater at 49.4 mm (over 5 times) compared to 2022's amount of only 7.5 mm. In August, the amount of precipitation in 2020 was over 2 times less than in 2020.

In 2022, the lowest average yield was obtained (859.7 kg/da) with a sum of rainfall during the growing season of 259.8 mm, while the yield in 2020 was 885.6 kg/da and the sum of rainfall (199.8 mm) was the lowest for the entire study period.

The yields of the tested maize hybrids over the years were influenced by the meteorological conditions during the growing season and especially during the period of pronounced formation of the reproductive organs; therefore, to enrich the discussion, we monitored the amounts of rainfall and the average daily temperature sum during the last ten days of June and the whole month of July, expressed by the

Selyaninov's hydrothermal coefficient (mentioned above). This coefficient helps to explain the results obtained in yields more thoroughly than the sum of rainfall over the whole growing season. For example, the highest average yield in 2023 is a consequence of a large amount of rainfall during the growing season. The next highest average yield is in 2021 and with the highest amount of rainfall (316.4 mm), but with an HTC of 0.36.

It is confirmed that the yields of the tested hybrids over the years depend on the weather conditions. Relating the yields to the values of the calculated HTC, it is evident that the highest results were obtained in 2023, when the sum of precipitation was 204.8 mm and the HTC during the generative period was 1.26, while the lowest yields were obtained from the hybrids in 2022, with a sum of precipitation during the growing season of 259.8 mm and the lowest HTC value of 0.15. In this experimental year, it is noteworthy the insignificant decrease in the yield of hybrid Kneja 310 (893.14 kg/da), compared to the average yield for the period (only 0.8%), while for the other hybrids the decrease was 3% for Kneja 461, 11% for Kneja 565 and 13.8% for Kneja 683A.

The productivity exhibited under specific climatic conditions during a particular period with different weather conditions can be estimated by the Sustainable Yield Index (SYI) calculated by a formula proposed by Singh et al. (1990). Though only a four-year study period, but with distinctly different agrometeorological conditions, we can estimate the yield stability of the tested hybrids. The reported values for this index reflected in Table 2 show the least deviation from the period average yield for hybrid Kneja 310 at 0.937, followed by the index of Kneja 461 at 0.841. With a lower stability index is hybrid Kneja 565 - 0.729 as a consequence of the larger range of yield variation from 893.4 kg/da in 2022 to 1168.6 kg/da in 2023. The lowest yield stability index is Kneja 683A - 0.685. Impressively, as the FAO maturity group advanced, the yield stability index decreased. This suggests that earlier hybrids have more stable yields. Our results are in agreement with Božović et al. (2020).

The analysis shows that the greatest influence on maize grain yields is the amount of rainfall during the generative stage.

From the bivariate analysis of variance (Table 3), it is evident that the largest proportion of the total variance (102618.8) is due to the conditions of the year. The specific genotypic responses exhibited by plants of different maize hybrids also showed high variation due to selection of different maturity group (FAO 300 to 600). The magnitude of the effect of the interaction of species and year traits was better manifested at $P<0.01$. Productivity was more determined by the factor of year conditions but also by the FAO group of the hybrid. The studied interaction of the two factors conditions of the year and hybrid appears to be a useful estimate of the adaptability of the hybrid depending on the realized productivity potential of its years of the period. A reliable interaction of year conditions and hybrid was found.

Table 3. Analysis of variance of the grain yield (ANOVA)

Source of Variation	SS	Df	MS	F	P-value	F crit	Factor influence
Year	307856.3	3	102618.8	24.8	8E-10	2.8	37.823
Hybrid	150371.4	3	50123.8	12.1	5E-06	2.8	18.474
Interaction	157277.3	9	17475.2	4.2	0.0005	2.1	
Within	198444.1	48	4134.25				
Total	813949.1	63				Error	43.703

The analysis shows that the greatest influence on maize grain yields is the amount of rainfall during the generative stage.

The two-factor analysis of variance applied to the maize hybrids tested revealed an interaction of the two factors (FAO conditions of the year and hybrid type) that significantly influenced maize productivity. The data showed a stronger influence of the year conditions factor compared to hybrid type on productivity.

CONCLUSIONS

The highest average grain yield over a four-year study period was shown by the hybrid Kneja 565 (1003.9 kg/da) with a high yield stability index SYI 0.729. Relatively high and stable yields were obtained from Kneja 310 (900.3 kg/da, SYI 0.937) and Kneja 461 (925.9 kg/da, SYI 0.841), respectively. The factor of year conditions had a stronger influence on grain yield of maize compared to hybrid.

In years with favorable conditions during the maize growing season, higher yields were obtained from hybrids in the mid- and late maturity groups, and in unfavorable years from those in the early and mid-early maturity groups.

ACKNOWLEDGEMENTS

This research work was carried out with the support of the Agricultural Academy – Sofia, Bulgaria.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

STUDY ON THE ADAPTABILITY OF SOME TRITICALE GENOTYPES TO DIFFERENT CLIMATE AND SOIL CONDITIONS

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Abstract

The paper aims to evaluate the adaptability of 25 triticale genotypes in distinct agro-climatic and pedological conditions using three experimental locations in Romania: ARDS Secuieni, ARDS Turda, and ARDS Piteşti. The study focused on determining the ability of genotypes to adapt to the variability of climatic and soil factors, emphasizing agronomic performance. The analyzed parameters included productivity, resistance to abiotic stress (drought, extreme temperatures), and the protein percentage of triticale grains. The results highlighted significant differences between genotypes in terms of adaptability to the specific conditions of each location, thus identifying genotypes with high potential for stability and productivity in variable environments. These data are relevant for selecting genotypes adapted to the agroecological diversity in Romania and contribute to developing sustainable triticale cultivation strategies.

Key words*: climatic condition, protein content, triticale, yield.*

INTRODUCTION

In recent years, all agricultural areas of Romania have been facing climate change, which significantly impacts agricultural production. Factors such as increasing temperatures, variations in precipitation, frequent droughts, and extreme weather events influence this.

The specialized literature highlights the interdependence between the main factors that influence agricultural production, emphasizing the close connection between biological material (seed, hybrid, variety, genotype, cultivar), the conditions of the crop area (relief, climate, soil), and the technology applied in the crop (Roman et al., 2011; Bîlteanu et al., 1979).

Triticale is distinguished by its ability to produce large quantities of grains and biomass compared to other grassy cereals, even in less favourable climatic and soil conditions. Due to its high adaptability, triticale can successfully replace rye and other cereals on sandy soils. It demonstrates good resistance to acidic soils with low pH and high tolerance to aluminium toxicity and drought. These characteristics have made triticale increasingly appreciated by farmers worldwide, and it is currently a crop in continuous expansion (Paiu, 2023).

In Romania, according to Gașpar and Butnaru (1985), cited by Mogârzan (2012), two cultivation zones are established for the triticale species. The first zone of favourability is represented by soils with low natural fertility, poorly supplied with mobile elements, and high acidity, where triticale ensures higher production increases than wheat, showing tolerance to diseases and the toxicity of aluminium ions.

The second cultivation area is represented by the agricultural land areas that are very favourable for the cultivation of wheat and

barley. In contrast, the triticale species find a less significant place (Mogârzan, 2012).
However, in recent times, due to climate change, the triticale species has expanded into all cultivation areas of Romania, capable of achieving higher production than other grassy cereals.

MATERIALS AND METHODS

To identify the influence of genotype, climate, and soil conditions on production and quality, 25 Romanian winter triticale cultivars were tested in three different crop areas in Romania: the Moldavian Plateau (ARDS Secuieni), the Transylvanian Plateau (ARDS Turda), and the Getic Plateau (ARDS Pitesti).
At A.R.D.S. Secuieni, the experience was located on a cambic chernozem soil type, weakly acidic (pH = 6.14), with a low humus content, poorly supplied with nitrogen, well supplied with phosphorus (77 ppm) and potassium (221 mg/kg) (Enea et al., 2023; Pochiscanu et al., 2017).
Analyzing the temperatures recorded during the vegetation period of triticale species, ARDS Secuieni recorded an average deviation of 5.1°C compared to the multiannual average (7.3°C) for the same period (Figure 1).

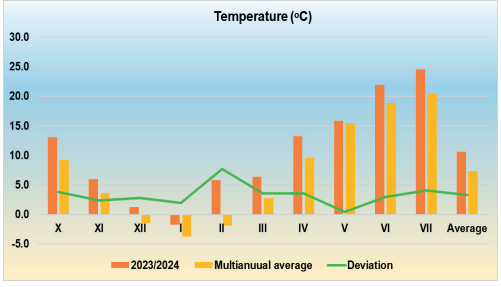


Figure 1. Monthly temperatures and multiannual average recorded at ARDS Secuieni during the vegetation period of the triticale species

February was the warmest month, recording an average temperature of 5.8°C compared to the multiannual average of -1.9°C. The average deviation of 7.7°C characterized February as warm, while May was characterized thermally as usual, with a deviation from the multiannual average of 0.4°C (Figure 1).
Figure 2 shows that at A.R.D.S. Secuieni, the analyzed period was unfavourable to the growth and development of the triticale species because there was a rainfall deficit compared to the multiannual average of -124.4 mm.
The lack of rainfall in the April-May period dried the siblings and pollen, resulting in lower production (Figure 2).

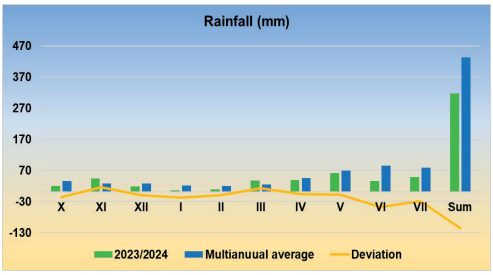


Figure 2. Monthly precipitation and multiannual average recorded at ARDS Secuieni during the vegetation period of the triticale species

At A.R.D.S. Turda, the soil on which the experiment was located is of the chernozem type characterized by an average content in humus (3.36-3.73%), moderate-good in total nitrogen (0.177-0.205%), poor-medium in mobile phosphorus (11-35 ppm) and very good in mobile potassium (220-320 ppm), and the soil reaction is slightly acidic to neutral (pH = 6.6.84) (Chețan et al., 2024).
At ARDS Turda, temperatures recorded during the triticale species' vegetation period deviated from the multiannual average of 3.5°C (Figure 3).

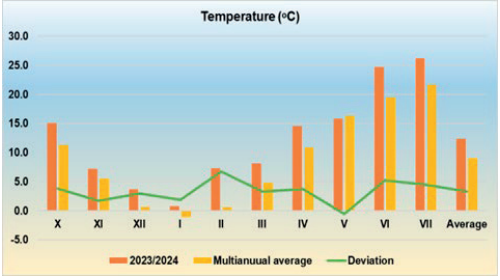


Figure 3. Monthly temperatures and multiannual average recorded at ARDS Turda during the vegetation period of the triticale species

February was the warmest month, with a deviation of 7.6°C compared to the multiannual average, which determined an accelerated start to crop vegetation (Figure 3).

The rainfall regime recorded at A.R.D.S. Turda was dry during the triticale species' vegetation period, with a deviation from the multiannual average of -116.3 mm (Figure 4).

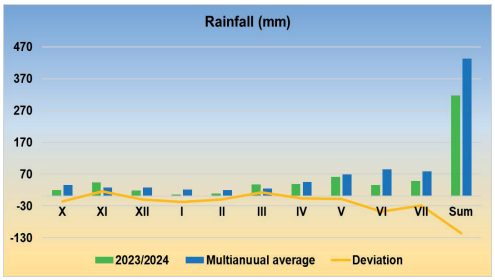


Figure 4. Monthly precipitation and multiannual average recorded at ARDS Turda during the vegetation period of the triticale species

Although precipitation exceeded the multiannual average recorded in November and March, their lack of critical phenophases caused the drying of the siblings and pollen.
The soil at ARDS Pitesti, on which the research was carried out, is a typical luvosol characterized as being poorly supplied with nitrogen (Nt = 0.130% mg/kg) and moderately supplied with potassium (KAL=89 mg/kg); poorly fertile, with a humus content in the arable horizon of 1.96%; acidic, with pH values (in aqueous suspension) of 5.02 (Gheorghe and Ghiorghe, 2024).
At ARDS Pitesti, during the analyzed period, an increase in average monthly temperatures of 3.3°C was recorded compared to the multiannual average (9°C), characterizing the vegetation period of the triticale species as very warm (Figure 5).

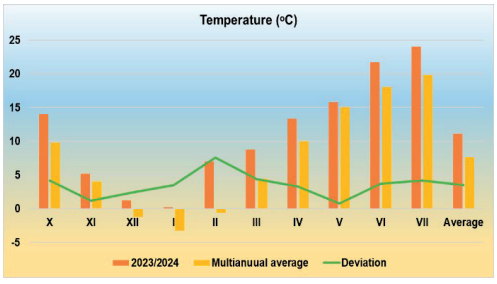


Figure 5. Monthly temperatures and multiannual average recorded at ARDS Pitesti during the vegetation period of the triticale species

As in the other two crop areas, February was the warmest month, with a deviation from the multiannual average of 6.7°C (Figure 5).
Precipitation recorded during the triticale growing season at ARDS Pitești had a negative impact on plant growth and development, with a deviation of -246.2 mm compared to the multiannual average (570.5 mm) (Figure 6).

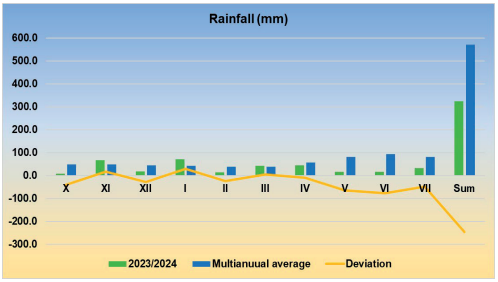


Figure 6. Monthly precipitation and multiannual average recorded at ARDS Pitesti during the vegetation period of the triticale species

The prolonged lack of precipitation starting in April led to a severe drought, causing crop plants to go through the development stages rapidly.
The Utrifun variety was chosen as a control due to its genetic stability, constant agronomic behavior, popularity in the tested crop areas, specific characteristics, and ability to reduce experimental variability. Thus, it provides a reliable reference point for comparing the efficacy and performance of other triticale genotypes.
The results were processed using the variance analysis method.

RESULTS AND DISCUSSIONS

Climate and soil conditions influenced the studied triticale genotypes. The results provide insight into their behaviour in various agroecological conditions, contributing to selecting the most suitable variants for cultivation in the targeted areas.
Thus, under the conditions at ARDS Secuieni, the yields of the studied genotypes ranged between 4767 kg/ha (Negoiu) and 8390 kg/ha (17241T1) (Figure 7).

When the yields obtained by the triticale genotypes were compared with the control, the Utrifun variety, at ARDS Secuieni, the Haiduc and Negoiu varieties obtained yields increases statistically interpreted as negatively significant and negatively distinctly significant, with yields ranging between 4767 kg/ha and 5042 kg/ha (Figure 7).
Yield increases were statistically interpreted as significant at the genotypes 18013T, 18061T, 18339T, and 07320T1 (Figure 7).
The genotypes 16512T2, 16026T1, 17300T1, and 18031T achieved average yields ranging between 7375 kg/ha and 7492 kg/ha. The yield increases were statistically interpreted as distinctly significant compared to the control (Figure 7).
Under the ecological conditions at ARDS Secuieni, the best results were obtained by the Zvelt genotypes, with an average yield of 7933 kg/ha and 17241T1, with an average output of 8390 kg/ha. Compared to the control, the yield increases obtained by these genotypes were statistically interpreted as being very significant (Figure 7).

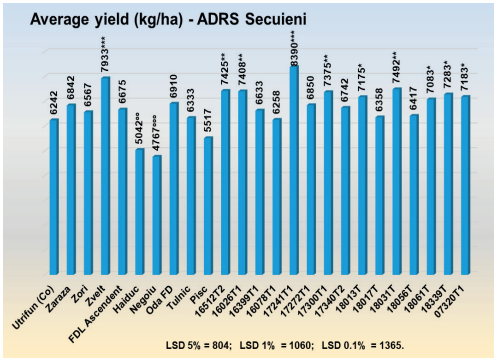


Figure 7. Average yield recorded at ARDS Secuieni

Under the conditions at ARDS Turda, the average yields obtained by the 25 triticale genotypes had values ranging between 5625 kg/ha (Tulnic) and 10158 kg/ha (18061T) (Figure 8).
The yield increases of the Tulnic and Haiduc varieties were statistically interpreted as being negatively significant (5787 kg/ha) and negatively distinctly significant (5625 kg/ha) compared to the experimental control (Figure 8).

Of the total triticale genotypes studied, 12 varieties achieved yield increases statistically interpreted as being very significant (Zaraza, 16512T2, 16399T1, 16078T1, 17241T1, 17272T1, 18013T, 18017T, 18031T, 18056T, 18061T, 18339T) compared to the control (Figure 8).

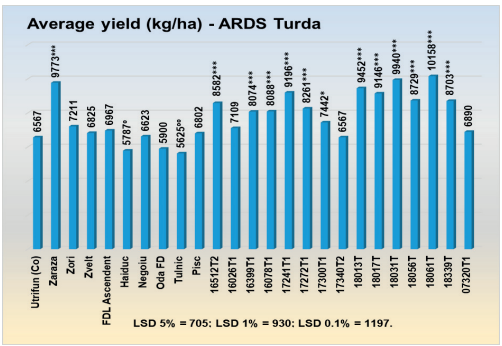


Figure 8. Average yield recorded at ARDS Turda

The agro-climatic conditions of 2024 were not favorable for triticale species under the conditions of ARDS Pitești.
Of the three crop areas, the area of influence of ARDS Pitești was droughty, with a deviation from the multiannual average of -246.2 mm (Figure 6).
The average yields obtained at ARDS Pitești were between 5133 kg/ha (18056T) and 6935 kg/ha (Zvelt) (Figure 9).
Figure 9 shows that most of the genotypes studied achieved yield increases statistically interpreted as negatively very significant compared to the control, the Utrifun variety, which achieved an average yield of 6838 kg/ha.

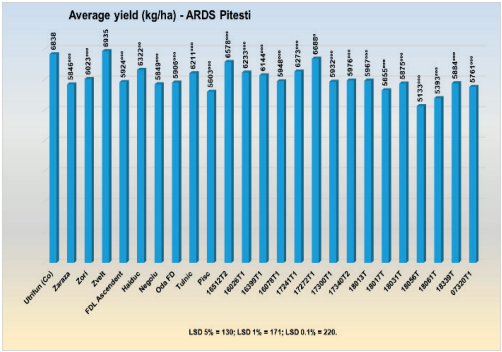


Figure 9. Average yield recorded at ARDS Pitesti

On average, the triticale genotypes studied in the three crop areas achieved yields ranging between 5717 kg/ha (Haiduc) and 7953 kg/ha (17241T1) (Figure 10).
The triticale varieties Haiduc and Negoiu achieved yield increases statistically interpreted as negatively very significant (Figure 10).

Higher yield increases than the control, statistically interpreted as highly significant, were obtained in the variants sown with the genotypes Zaraza, 16512T2, 17241T1, 18013T, 18031T, and 18061T (Figure 10).

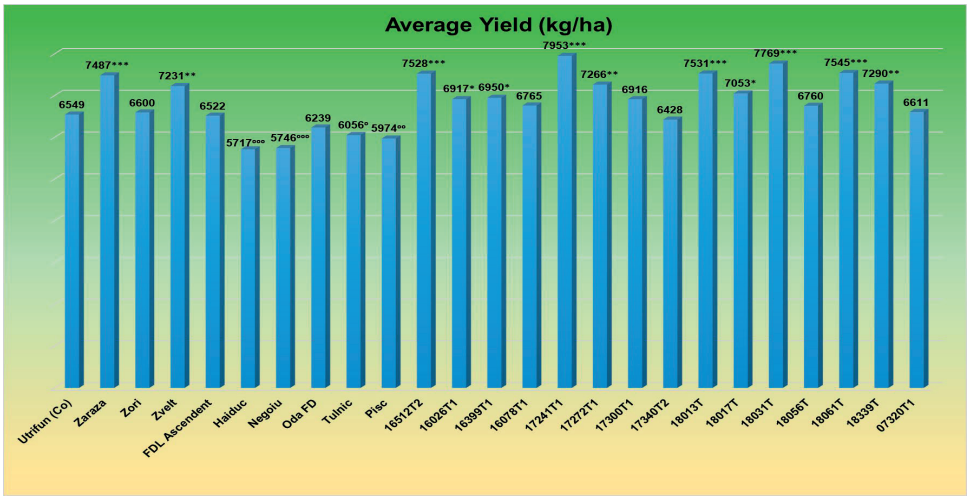


Figure 10. The average yield achieved by the studied triticale genotypes

Protein content is an important indicator of the quality of agricultural products.
Thus, the studied genotypes responded differently depending on the pedo-climatic cultivation conditions.
In the three test centers, the protein content was between 14.6% (16078T1) and 17% (18056T) at ARDS Secuieni, between 9.4% (07320T1) and 11.3% (Tulnic) at ARDS Turda and between 12.4% (Zaraza) and 15.3% (Zvelt) at ARDS Pitesti (Figure 11).
The studied genotypes achieved an average protein content between 12.5% (Zaraza, 07320T1) and 14% (18056T) (Figure 11).

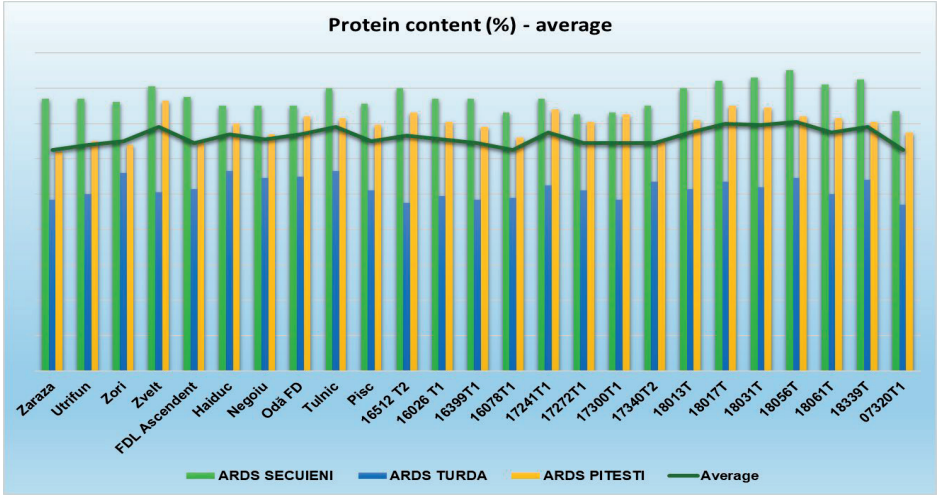


Figure 11. The average protein content obtained by the studied genotypes

CONCLUSIONS

Following the above, we can conclude that drought is occurring in the three crop areas due to high temperatures, which negatively influence crop plant growth and development.
Based on the results, the new triticale genotypes stand out for their high yield capacity. Among the genotypes studied, the 17241T1 line stands out, which achieved an average yield of 7953 kg/ha.
Line 18056 T recorded the maximum average protein content (14.1), and the minimum was recorded in the case of variants sown with the Zaraza variety (12.5%).

ACKNOWLEDGEMENTS

This research was supported by a grant from the Ministry of Agriculture and Rural Development as part of the National Research-Development Plan in the field of Agriculture and Rural Development, "Agriculture and Rural Development – ADER 2026", project no. 1.1.3.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

SUGAR BEET PEST DYNAMICS IN THE CONDITIONS OF CENTRAL MOLDOVA (ROMANIA)

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Abstract

The plant's development near the soil characterizes the sugar beet agrosystem. Soil pests are important in crop evolution, and less so the foliar ones. Research carried out at the Agricultural Research and Development Station Secuieni Neamț consisted of inventorying the species that attacked sugar beet plants from the emergence period to the formation of the leaf rosette. The results showed that eight species attacked the sugar beet crop, belonging to the Coleoptera, Lepidoptera, and Homoptera orders. The highest densities of pests specific to sugar beet crops were recorded for the species Chaetocnema spp. (30 specimens), Bothynoderes punctiventris L. (26 specimens) and Opatrum sabulosum L. (18 specimens). In sugar beet crops, attacks by Agriotes larvae at seed germination were 30%, and during the vegetation period, the plants were attacked by B. punctiventris L. and Tanymecus spp. (30%), Chaetocnema spp. (15%), A. gamma L. (9%) and Aphis fabae (23%).

Key words*: sugar beet, attack, entomofauna, climatic conditions.*

INTRODUCTION

Sugar beet is mainly used as a raw material in the sugar industry, and the molasses resulting from its processing is used in the food and alcohol industries. A wide range of pathogens and pests that affect plants throughout the growing season can quantitatively and qualitatively reduce sugar beet yield (Baicu and Săvescu, 1978).

In Romania, the area cultivated with sugar beet was 21,330 thousand ha in 2020 and decreased to 19,640 thousand ha in 2021, respectively, to 8,890 thousand ha in 2022 (FAO STAT, 2024).

The sugar beet agrosystem has as a particularity the development of the plant in and near the soil. Many pests, especially monophagous and oligophagous, have migrated from Chenopodiaceae weeds, part of the same family as sugar beet, forming a specific entomofauna. They were accompanied by entomophagous. Some species found an abundant source of food in sugar beet crops and began to prefer this crop (Baicu and Săvescu, 1986).

Ciochia et al. (1984) mention 13 pathogens that appear in sugar beet crops from germination to harvest. The harmful entomofauna comprises 37 specific and polyphagous pests that can attack plants either in the adult or larval stage or in both stages. Some are vectors that infect plants with pathogens.

However, the highest sensitivity of sugar beet is to attack by soil and plant pests during the period of seed germination - plant emergence - complete formation of the leaf rosette (Cantar, 1997; Cantar et al., 1997; Rășcănescu et al., 1997).

The scientific literature indicates that the most well-known species of pests of sugar beet are *B. punctiventris* and *Tanymecus* spp., whose populations have experienced an expansion that occurred due to the increase in average temperature and changes in the control methods of these insects (Drmic et al., 2018). From 1990 to 2018, pest populations and their attack were controlled with neonicotinoid insecticides used in chemical seed treatment, and foliar application of insecticides was made only when necessary to combat soil pests when their densities exceeded the Economic Damage Threshold (Bažok et al., 2012). Therefore,

neonicotinoids contributed to a significant reduction in the amount of insecticide used in sugar beet cultivation. Treating sugar beet seeds with imidacloprid has been confirmed to protect young plants against low to moderate infestation with wireworms, fleas, and aphids (Igrc et al., 2000). Viric Gasparic et al. (2020) showed that the treatment of sugar beet seeds with neonicotinoid insecticides leaves minimal traces in the plants due to complete degradation, but also that they reduced the attack of soil pests and provided good protection to beet plants (Viric Gasparic et al., 2021).

The withdrawal of neonicotinoids from the chemical treatment of beet seeds led to the rapid appearance and spread of aphids in the crop, the attack being at a rate of 100%. This attack recorded in 2020 in Western European countries occurred during a very mild winter that led to high overwinter survival of the primary vector, the aphid *Myzus persicae*, which migrated from the hosts where they overwintered to those that emerged in April, i.e., in beet crops (Dewar and Qi, 2021).

The paper presents results obtained between 2020-2024 regarding the inventory of harmful organisms and attacks on sugar beet crops in Central Moldova (Romania) without applying chemical treatments to control damaging insects.

MATERIALS AND METHODS

***Location*:** The research was conducted at the Agricultural Research and Development Station Secuieni - Neamț (Romania), located at 26°5' east longitude and 46°5' north latitude, in a hilly area where altitudes rise to 250 m above sea level (a.s.l.). The region's topography includes extensive interfluve plains, grasslands, and terraces (Trotuș et al., 2020). The area has a temperate continental climate, D.f.b. in the updated Koppen-Geiger climate classification, with short springs, cool summers, and harsh winters (Kottek et al., 2006).

The experiments with sugar beet were located in the experimental field of the Plant Protection Laboratory, on a typical cambic chernozem soil, with a pH in water of 6.29, a humus content of 2.3, a nitrogen index of 2.1, a mobile P_2O_5 content of 39 ppm and a K_2O content of 161 ppm.

Observation methodology: Monitoring pests' occurrence, spread, and evolution was carried out periodically through soil surveys with a 25/25 cm metric frame and collections using Barber traps installed in the field between plant emergence and crop harvest. During the vegetation period of the sugar beet crop, observations were made regarding the attack produced by specific harmful organisms. Determinations regarding the attack produced by pests were made through visual analysis of the plants (25 plants x 10 repetitions). The scores were made by giving grades according to the 0-6 scale (0 = no attack; 1 = 1-3% attack; 2 = 4-12% attack; 3 = 13-25%; 4 = over 26-50% attack; 5 = 51-75%; 6 = over 76%). Each species' frequency, intensity, and degree of attack were calculated based on the grades given (Pintilie et al., 2023).

The frequency of attack (F%) is the ratio between the number of attacked plants or organs of the attacked plant (n), related to the number of plants or vegetative organs observed (N): F% = (n x 100)/N.

Attack intensity (I%) represents, in fact, the percentage of attacked plants or organs of the plant destroyed by the pest: I% = (i x f)/n.

The degree of attack (DA%) is the product of these two indicators: DA% = (F% x I%)/100.

This paper used the attack frequency values for *Agriotes* spp. and *Aphis fabae*, respectively, for the remaining species and the degree of attack values.

No chemical treatments were applied to the sugar beet crops to control harmful insects.

Climatic conditions: Climatic data were recorded using a Wireless Vantage Pro 2 Plus weather station (SC Rom Tech SRL, Sibiu, Romania) near the experimental field. To characterize the years from a climatic point of view, we used data on the average air temperature (◦C) recorded at 2 m height and the amount of rainfall (mm).

RESULTS AND DISCUSSIONS

Climatic conditions

Monthly temperatures were much higher during the analyzed period than the multiannual average (Figure 1).

The spring months were characterized by atypical temperatures, with periods in which

recorded high temperatures alternated with intervals of low temperatures. The summer months were warm and very warm from a thermal point of view in the years in which the research was carried out (Figure 1). These conditions determined plants' faster growth and development, with temperatures recorded on average 4.3°C higher than the multiannual average.

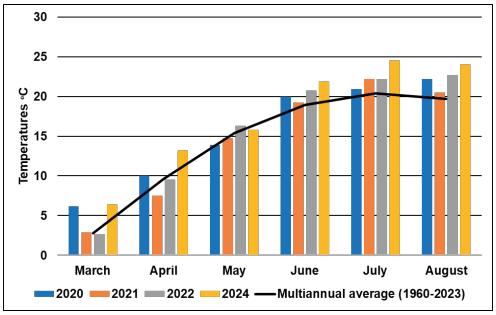


Figure 1. Average monthly temperatures recorded between March and August, Secuieni - Neamț

In terms of rainfall, the analyzed period recorded a rainfall deficit each year in which the research was carried out.

The spring months were deficient in rainfall, which raised problems in soil preparation and sowing of the sugar beet crop. This negatively influenced the emergence of plants, which occurred unevenly and staggered, and the growth and development of plants were slow (Figure 2).

The summer months, except June, when precipitation amounts close to the multiannual average were recorded, which helped the beet plants to develop, were deficient in rainfall, which negatively influenced the evolution of the crop and led to the qualitative and quantitative depreciation of the harvest (Figure 2).

Therefore, the climatic data recorded at the meteorological station of ARDS Secuieni presents an increase in average air temperatures and a decrease in rainfall during the spring and summer months (Isticioaia et al., 2020). An earlier spring-summer period is noted, which leads to the appearance, spread, and early attack of harmful organisms. Also, the lack of rainfall leads to uneven crop emergence, and extreme summer temperatures accelerate crop development. The plants reached maturity faster, and the yield was small and of poor quality.

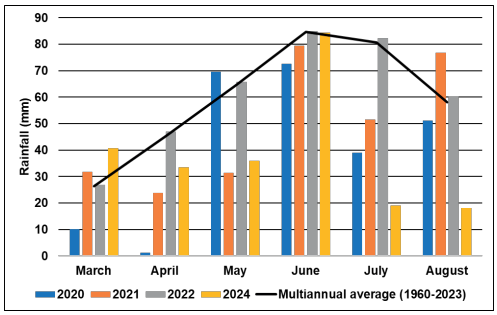


Figure 2. Monthly rainfall recorded between March and August, Secuieni - Neamț

Entomofauna in beet crops

From 2020 to 2024, the entomofauna inventoried in sugar beet crops consisted of eight insect species. The number of specimens collected varied from one year to another, with 80 specimens/m^2 identified in 2020 along with seven aphid colonies, 64 specimens/m^2 recorded in 2021, 64 specimens/m^2 recorded in 2022, 64 specimens/m^2 identified in 2022 along with five aphid colonies, and 196 specimens/m^2 and 11 colonies identified in 2024 (Table 1). By species, the most numerous specimens belonged to the *Chaectocnema* spp., with 30 specimens, followed by *B. punctiventris* with 26 specimens, and *O. sabulosum* with 18 specimens (Table 1).

Table 1. Harmful entomofauna to sugar beet crops inventoried in 2020 - 2024, Secuieni - Neamț

No.	Order/Species	Density specimens/m^2				Average
		2020	2021	2022	2024	
Coleoptera						
1	*Agriotes spp*	8	4	4	5	5
2	*Tanymecus spp.*	26	14	5	11	14
3	*Opatrum sabulosum* L.	0	4	0	66	18
4	*Gonocephalum pusillum* Fabricius	1	3	6	3	3
5	*Bothynoderes punctiventris* L.	11	9	16	67	26
6	*Chaectocnema spp*	29	26	33	33	30
Lepidoptera						
7	*Autographa gamma* L.	5	4	0	11	5
Homoptera						
8	*Aphis fabae* Scopoli	7 col	0	5 col	11 col	6 col
Total = three orders/eight species		80 + 7 *	64	64 + 5 *	196 + 11*	101 + 6 *

*colony

Calculating species share according to the number of specimens collected, we find that *Chaectocnema* spp. recorded the highest value, 28%, followed by *B. punctiventris*, with 24%, *O. sabulosum*, with 17%, *Tanymecus* spp., with 13%, *A. fabae, Agriotes* spp, and *A. gamma*, with 5% each, and *G. pusillum*, with 3% (Figure 3).

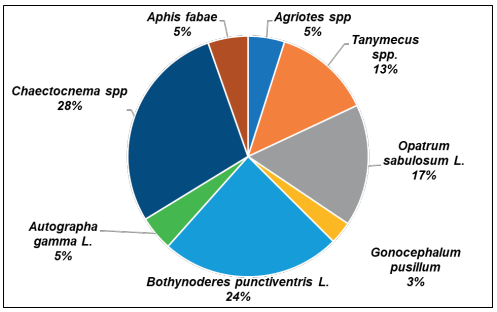


Figure 3. Species share according to the number of specimens collected, Secuieni – Neamț

Following the determinations carried out in the vegetation period, during the period 2020-2024, we found that *Agriotes* larvae were present and caused attacks in the sugar beet crop. The *Agriotes* larvae fed on the germinating seeds, which reduced plant density and gaps in the crop, and the yield potential was affected from the beginning of the plant emergence (Popov et al., 2001).

The frequency of attack by the larvae of the *Agriotes* species on grain was, on average, 30%. The highest attacks produced by the larvae on grain was recorded in 2020, at 37%, and the lowest attack was in 2021, at 19% (Figure 4).

Regarding the attack caused by the *Agriotes* larvae on the plant, the frequency of attacked plants was, on average, of 1%, and over the years, it varied between 0% (2024) and 3% (2021) (Figure 4).

The percentage of plants saved was, on average, 69%, recording values between 60% (2022) and 78% (2021) (Figure 4).

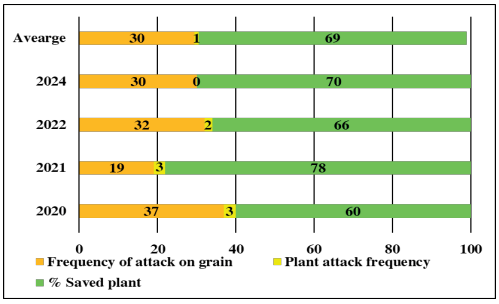


Figure 4. Frequency of attack by *Agriotes* spp., Secuieni - Neamț

In addition to the attack caused by the *Agriotes* species, the damage caused by the rest of the species is also added: *B. punctiventris* L., *Tanymecus* spp., *Chaetocnema* spp., *A. gamma* L. and *A. fabae*.

The fleas appeared as the plants emerged. They consumed the leaves' epidermis, the attack being recognized by the appearance of translucent holes. The weevils' adults and larvae consumed the young leaves, leaving only the petiole (Figure 5).

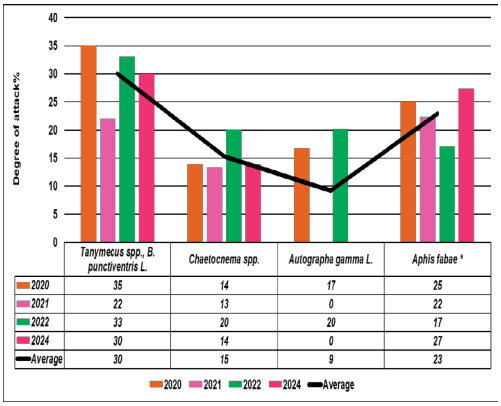


Figure 5. Attack caused by specific pests on beet: *Chaectocnema* spp. (left) and *B. punctiventris* (right) (original photo)

The attack of specific pests was recorded through determinations carried out in the vegetation, and we found that:

- in 2020, five species of harmful insects attacked the crop: the species *B. punctiventris* L. and *Tanymecus* spp. recorded the highest degree of attack rates, of 35%, followed by *A. fabae* with a frequency of attack of 23% (Figure 6);
- in 2021, the attack was lower because the spring conditions characterized the cool and dry period, the attack of *Autographa* larvae was no longer recorded, and the rest of the species recording degrees of attack values between 13% (*Chaectocnema* spp) and 22% (*B. punctiventris* L. and *Tanymecus* spp.) (Figure 6);
- in 2022, the conditions were favorable for the species attack, the degree of attack being between 20% (*A. gamma* L.) and 33% (*B. punctiventris* L. and *Tanymecus* spp.) (Figure 6);
- in 2024, the highest degree of attack was recorded by *B. punctiventris* L. and *Tanymecus* spp., at 30%, followed by *A. fabae,* which, following the determinations carried out, had values of the frequency of attacked plants of 27% (Figure 6);
- on average, *B. punctiventris* L. and *Tanymecus* spp. recorded the highest degree of attack values of 30%, followed by *A. fabae*, where the

frequency of attacked plants was 27%, and the lowest attack rate values of 9% were recorded at *A. gamma* (Figure 6).



	Tanymecus spp., B. punctiventris L.	Chaetocnema spp.	Autographa gamma L.	Aphis fabae *
2020	35	14	17	25
2021	22	13	0	22
2022	33	20	20	17
2024	30	14	0	27
Average	30	15	9	23

Figure 6. Degree of attack by specific pests, Secuieni – Neamț (*- values of frequency of attacked plants)

It was found that sugar beet plants were attacked by aphids at a high frequency each year, ranging between 17% and 27%, which requires the application of corrective treatments to control the spread of aphids (Figure 7).



Figure 7. *A. fabae* colonies on sugar beet

The species identified in sugar beet crops are the most widespread and produce the highest attacks if climatic conditions are favorable (Doncilă, 2006).

Our results were obtained without applying chemical seed treatment and systemic insecticides on vegetation. They showed that soil pests produced a high degree of attack and that the identified species had favorable conditions for emergence, spread, and attack.

CONCLUSIONS

The insect fauna that was harmful to the sugar beet crop from the emergence period until the formation of the leaf rosette was composed of eight species belonging to the orders Coleoptera, Lepidoptera, and Homoptera.

The highest densities of pests specific to the sugar beet crop were recorded at *Chaetocnema* spp. (30 specimens), *B. punctiventris* (26 specimens) and *O. sabulosum* (18 specimens).

In sugar beet crops, attacks were registered on the grain by *Agriotes* (30%) and on the plants during the vegetation period by *B. punctiventris* and *Tanymecus* spp. (30%), *Chaetocnema* spp. (15%), *A. gamma* (9%) and *A. fabae* (23%).

ACKNOWLEDGEMENTS

This work was prepared within the framework of the ADER 2023 Project no. 5.1.6/18.07.2023, "Research on the establishment of low-input control systems for major pests in beet and potato crops through the use of phytosanitary products with reduced environmental impact" funded by the Romanian Ministry of Agriculture, Rural Development and Rural Development.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

ASSESSMENT OF THE THERAPEUTIC POTENTIAL APPLICATION OF THE EXTRACTS FROM DIOECIOUS HEMP (*Cannabis sativa* L.)

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Abstract

Dioecious hemp (Cannabis sativa L.) is an industrial crop utilised for various purposes, including fibre, seeds, and plant extracts. The seeds and plant extracts are used for food, pharmaceuticals, or cosmetic products. This study explores the therapeutic potential of dioecious hemp by analysing the antibacterial activity of hydroalcoholic extracts from four varieties of CS: CS 1 - Armanca, CS 2 - Lovrin 110, CS 3 - Silvana, and CS 4 - Teodora. The antibacterial properties of the examined extracts were evaluated against two clinically significant ATCC bacterial strains: Staphylococcus aureus (ATCC 25923) and Escherichia coli (ATCC 25922). The main parameters assessed included bacterial growth percentage (BGP%) and bacterial inhibition percentage (BIP%), and minimum inhibitory concentration (MIC). These were utilised to evaluate the effectiveness of the extracts. The findings indicated a positive correlation between the concentration of CS extract and its antibacterial activity. Specifically, higher extract concentrations resulted in stronger inhibitory effects on bacterial growth. However, further research is crucial to fully understand the potential of hemp hydroalcoholic extracts as components in antibacterial pharmaceutical products.

Key words: *dioecious hemp extracts, antibacterial activity, BGP, BIP, MIC, hydroalcoholic extracts.*

INTRODUCTION

Dioecious hemp (*Cannabis sativa* L.) is a versatile plant prized for its industrial, nutritional, and therapeutic uses (Andre et al., 2016). As a dioecious species, *C. sativa* produces separate male and female plants, each contributing uniquely to its chemical profile (Small et al., 2007). Cannabis is extensively grown globally and has a rich cultivation history in China, valued for its uses in textiles and consumption. Its historical uses for fibres, seeds, and medicinal properties, as well as contemporary studies concentrating on its bioactive compounds, connect us to a rich heritage (Gallily et al., 2015).

The pharmacological potential of *C. sativa* arises from its complex chemical composition, which encompasses over 565 natural compounds, prominently 120 cannabinoids along with terpenoids and flavonoids (ElSohly & Slade, 2005). Cannabinoids, particularly cannabidiol (CBD) and tetrahydrocannabinol (THC), demonstrate diverse biological activities, including antimicrobial effects (Appendino et al., 2008). A recent review highlighted the uses of *C. sativa* from ancient times to today, covering ethnological, botanical, chemical, and pharmacological aspects, emphasising pharmaceutical research to illustrate the plant's potential therapeutic benefits (Bonini et al., 2018). Particularly, the morphological components, like the inflorescence, are crucial because they contain potentially biologically active compounds (Richins et al., 2018; Tiago et al., 2022).

As antimicrobial resistance continues to rise globally, *C. sativa* extracts are gaining interest for their antibacterial effects. Research demonstrates the effectiveness of hydroalcoholic extracts against *Staphylococcus aureus* and *Escherichia coli*, showing that inhibitory activity increases with higher extract concentrations (Blázquez & Bermejo, 2018). Moreover, root extracts from *C. sativa* demonstrate antioxidant and bactericidal effects, reinforcing its therapeutic importance (Pollastro et al., 2018). *E. coli* poses a significant threat to the healthy growth of both humans and animals (Lagha et al., 2019). In certain circumstances, it can lead to diarrhea in humans and animals (Yue et al., 2020). Additionally, it can cause food

spoilage, which is a central concern in food preservation (Gutiérrez-del-Río et al., 2018). *S. aureus* is a Gram-positive, aerobic bacterium that acts as an opportunistic pathogen (Zamora-Mendoza et al., 2022). This bacterium primarily colonises the skin and mucous membranes. It can lead to diseases ranging from common skin infections to more severe conditions such as endocarditis, bloodstream infections, and osteomyelitis (Jenul & Horswill, 2019).

Numerous studies indicate cannabidiol (CBD) may possess antimicrobial properties, particularly against drug-resistant *S. aureus* strains (Martinenghi et al., 2020; Ferenczy et al., 1958; Appendino et al., 2008). Both cannabis extracts and isolated cannabinoids have demonstrated greater effectiveness against Gram-positive bacteria, including multidrug-resistant species (Schofs et al., 2021). Although there are promising results, the antimicrobial capabilities of certain *C. sativa* varieties have still not been thoroughly researched. This study assesses the antibacterial effects of hydroalcoholic extracts from four dioecious varieties against *S. aureus* and *E. coli*, aiming to aid in the creation of new antibacterial agents derived from *C. sativa*.

MATERIALS AND METHODS

The study was conducted as a field experiment during the 2023 growing season at the Agricultural Research Station of University of Life Sciences "King Mihai I" from Timisoara, Romania. This location was chosen due to its conducive climate and soil conditions for hemp cultivation, providing an ideal setting for the study. A randomised block design utilised four dioecious *Cannabis sativa* L. varieties as the experimental factor: CS1 - Armanca, CS2 - Lovrin 110, CS3 - Silvana, and CS4 - Teodora. Each variety was replicated three times, with plots measuring 10 m² (Figure 1). Rows were spaced 70 cm apart, while plants in a row were 50 cm apart. To promote optimal growth conditions, seeds were sown manually. Uniform agronomic methods were applied across all plots. Female inflorescences were harvested at full bloom. The collected material was air-dried in a shaded area at room temperature (around 22°C) to maintain bioactive compounds. Using a laboratory mill, the dried material was then ground into a fine powder. For extraction, 1 g of the plant material is mixed with 10 ml of 70% ethanol for 30 minutes with vigorous shaking. The resulting extracts are filtered through high porosity filter paper. After extraction, the samples were diluted to a ratio of 1:100 with 70% ethanol.



Figure 1. Four dioecious *Cannabis sativa* L. varieties

Microbiological method: A 10^{-3} dilution of *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922) strains were used. The cultures were then diluted to an optical density (OD) of 0.5 McFarland standard (1,5 × 10^8UFC×mL) using Brain Heart Infusion (BHI) broth (Oxoid, CM1135) and assessed with a McFarland densitometer (Grand-Bio, England). Subsequently, 100 µl of the dilutions were

pipetted into each well of a 96-well microdilution plate using a Calibra 852 digital multichannel pipette.

Test extracts were added in volumes of 25 µL, 50 µL, 75 µL, and 100 µl. The plates were covered and incubated for 24 hours at 37°C. After this period, the optical density (OD) was measured at 540 nm using an ELISA reader (BIORAD PR 1100, Hercules, CA, USA). All samples were tested in triplicate. For the positive control, strain suspensions in BHI were utilised. The broth microdilution method is a fundamental technique for antimicrobial susceptibility testing (Clinical and Laboratory Standards Institute, 2017, Methods for Dilution Antimicrobial Susceptibility Test for Bacteria that Grow Aerobically: Approved Standard-8th Edition, CLSI Document M07-A8, Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2009. Medicines, 4, 58). This method involves double dilutions of the antimicrobial agent in a liquid culture medium, which is organised in 96-well microtiter plates. The minimum inhibitory concentration (MIC) is defined as the lowest concentration of the antimicrobial agent that prevents the growth of the organism. CLSI has standardised the broth microdilution method for aerobically growing bacteria, yeasts, and filamentous fungi.

The EUCAST microdilution method closely resembles the CLSI, but it usually includes modifications related to specific test parameters like inoculum preparation, size, and MIC reading.

RESULTS AND DISCUSSIONS

Results are shown as bacterial growth percentage (BGP%) and bacterial inhibition percentage (BIP%). These percentages are calculated using formulas (1) and (2):

$$BGP\% = OD_{sample}/OD_{negative\ control} \times 100 \quad (1)$$

$$BIP\% = 100 - BGP \quad (2)$$

In all tested extracts, bacterial growth percentages presented a negative correlation with the increasing concentration tested as presented in Figure 2 and Figure 3.

Figure 2 presents a graphical representation of the efficacy of CS extracts against *S. aureus* expressed as BGP% and BIP% while Figure 3, represents graphically the activity of CS extracts against *E. coli*.

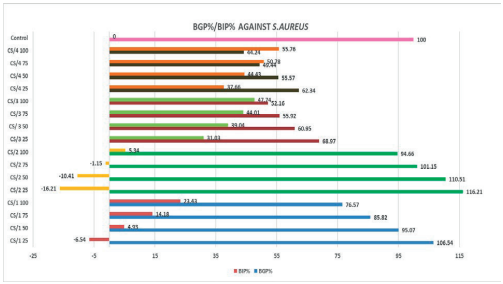


Figure 2. Graphical representation of the activity of CS extracts against *Staphylococcus aureus* expressed as bacterial growth percentage (BGP %) and bacterial inhibition percentage (BIP %)

The antibacterial activity of CS1 - Armanca extract is mild to moderate and rises with increasing dosage.

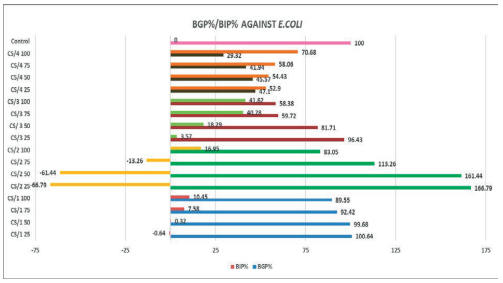


Figure 3. Graphical representation of the activity of CS extracts against *Escherichia coli* expressed as bacterial growth percentage (BGP %) and bacterial inhibition percentage (BIP %)

When it comes to *S. aureus*, the bacterial growth percentage (BGP %) decreases from 106.54% at 25 µL to 76.57% at 100 µL, while the bacterial inhibition percentage (BIP %) increases from -6.54% to 23.43%. This indicates an inhibitory effect that is dose-dependent. While less noticeable, a similar pattern is seen when looking at *E. coli*, BIP % increases from -0.64% to 10.45%, while BGP % decreases from 100.64% to 89.55%. Slightly more effective against *S. aureus* than *E. coli*, CS1 - Armanca exhibits a moderate inhibitory effect overall.

Considering the bacterial growth CS2 - Lovrin 110 exhibits inconsistent and generally weak antibacterial effects. At lower doses (116.21% at 25 µL), the BGP% for *S. aureus* exceeds 100%, suggesting enhanced bacterial growth as opposed to inhibition. The BGP % only decreases below 100% at 100 µL, when it reaches 94.66% (BIP % = 5.34%). The impact

on *E. coli* is more problematic: at 25 µL, BGP % raises significantly to 166.79% and remains higher than 100% up until the highest dose, at which point it decreases to 83.05% (BIP % = 16.95%). These findings suggest that CS2 - Lovrin 110 may only slightly inhibit bacterial growth at higher volumes and could actually stimulate bacterial growth at lower doses, especially for *E. coli*.

Strong antibacterial activity is shown by CS3 - Silvana, which steadily becomes more effective as the volume increases. BIP% values for *S. aureus* increase from 31.03% to 47.74%, while BGP % decreases from 68.97% at 25 µL to 52.16% at 100 µL. Similarly positive outcomes are observed for *E. coli:* BIP % increases from 3.57% to 41.62% while BGP % decreases from 96.43% to 58.38%. CS3 - Silvana is a highly effective treatment, especially noticeable for its balanced action against both *S. aureus* and *E. coli*. These results indicate an effective, dose-dependent antibacterial effect against both bacterial strains.

Among the tested samples, CS4 - Teodora has the strongest antibacterial activity, especially against *E. coli*. Strong inhibition is indicated according to the BGP % for *S. aureus*, which decreases from 62.34% at 25 µL to 44.24% at 100 µL (BIP % = 37.66% to 55.76%). The effect is even more noticeable against *E. coli*: BIP % ranges from 52.9% to 70.68%, while BGP% decreases from 47.1% to 29.32%. The data conclusively demonstrate that CS4 - Teodora inhibits bacterial growth in a potent, dose-dependent manner. It is particularly effective against *E. coli*, making it the most promising formulation tested for antibacterial use.

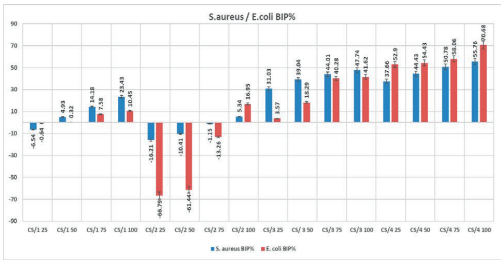


Figure 4. Graphical comparison of the CS efficacy against *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922)

Figure 4 displays the inhibition values of CS extracts against *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922) strains in comparison and Table 1 presents a summary of the CS efficacy at the highest concentration tested.

Table 1. Summary of the CS efficacy at the highest concentration tested

Sample	BIP% *S. aureus ATCC 25923*	BIP% *E. coli ATCC 25922*	Most Effective Against
CS/1	23.43	10.45	*S. aureus*
CS/2	5.34	16.95	Weak/Inconsistent
CS/3	47.74	41.62	Both, slightly *S. aureus*
CS/4	55.76	70.68	*E. coli*

BIP%-bacterial inhibition percentage

CS4 - Teodora proved to be the most effective antibacterial formulation according to the inhibition percentages at the highest tested volume (100 µL). It was especially effective against *E. coli* (BIP % = 70.68%) and also significantly inhibited *S. aureus* (BIP % = 55.76%). With significant inhibition of both strains (*S. aureus* = 47.74%, *E. coli* = 41.62%), CS3 - Silvana came in second. While CS2 - Lovrin 110 was the least effective and even stimulated bacterial growth at lower doses, particularly in *E. coli*. CS1 - Armanca demonstrated moderate inhibition and was more effective against *S. aureus* (23.43%) than *E. coli* (10.45%). This suggests that *S. aureus* is partially more responsive to treatment at moderate concentrations, whereas *E. coli* could require higher doses for noticeable inhibition.

The findings indicate a concentration-dependent antibacterial effect, with higher extract concentrations associated with increased bacterial inhibition rates (BIP %) and decreased bacterial growth rates (BGP %). Blázquez and Bermejo (2018) reported similar findings, emphasising the effectiveness of cannabinoids against resistant bacterial strains. The inhibitory activity observed can likely be attributed to cannabinoids and terpenoids in the extracts, which disrupt bacterial membranes and inhibit biofilm development (Appendino et al., 2008). Notably, *S. aureus*, a Gram-positive bacterium, showed greater susceptibility to the extracts than *E. coli*, a Gram-negative bacterium. This aligns with Pollastro et al. (2018), which connects bacterial cell wall composition with different sensitivities to plant-derived antimicrobials.

Additionally, the minimum inhibitory concentration (MIC) values in this study fall within the range documented for hydroalcoholic extracts of *C. sativa* (Russo, 2011), highlighting their potential as natural antibacterial agents. The intense activity of CS3 - Silvana and CS4- Teodora corresponds with ElSohly and Slade (2005), who observed that specific hemp cultivars display varying bioactivity due to differences in their phytochemical compositions. The analysis reveals that dioecious hemp plant extracts exhibit a negative correlation with the tested concentration increase and the bacterial growth cofactors.

CONCLUSIONS

In conclusion, the antibacterial effectiveness of the tested samples varied significantly across both bacterial strains. CS4 - Teodora demonstrated the strongest inhibitory activity, followed closely by CS3 - Silvana, with both showing clear dose-dependent effects. CS1 - Armanca exhibited moderate activity, while CS2 - Lovrin 110 was largely ineffective and sometimes stimulated bacterial growth. Overall, the treatments were generally more effective against *S. aureus* at lower concentrations, whereas *E. coli* required higher doses for comparable inhibition. These findings imply that various hemp varieties may possess unique antibacterial mechanisms and efficacies depending on the concentration of the extract. In summary, this research emphasises the therapeutic promise of dioecious hemp as a source of bioactive compounds that possess antibacterial qualities. The findings lay the groundwork for further investigation into creating hemp-derived antibacterial products and stress the necessity of choosing particular hemp varieties and extract concentrations to enhance effectiveness. Subsequent research should aim to clarify the underlying mechanisms of action and investigate more uses for these natural extracts in fighting bacterial infections.

ACKNOWLEDGEMENTS

This study was conducted with assistance from the Interdisciplinary Research Platform at University of Life Sciences “King Mihai I” from Timisoara, where all analyses occurred.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

TESTING THE EFFICACY OF ESSENTIAL OILS AGAINST *Drosophila suzukii* (Matsumura)

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Abstract

Drosophila suzukii (Matsumura) is an economically important pest of berry species. It is characterized by high reproductive potential and wide food specialization. For control of Drosophila suzukii, insecticides are mainly used, which lead to the development of resistance, therefore other alternatives are sought. In this regard, the efficacy of essential oils of lavender (Lavandula angustifolia Mill.), rosemary (Rosmarinus oficinalis L.) and peppermint (Mentha piperita L.) against adults of Drosophila suzukii was tested in two concentrations 0.1% and 0.2%. The study was carried out under laboratory conditions. The processing of the obtained data was carried out with a package of statistical programs Statistika 7 V. The highest efficacy was reported for rosemary, followed by mint and lavender. A gradual increase in efficacy was observed for all essential oils. Regression analysis of the results shows functional relationships between the concentration of the working solution and efficacy. As the concentration of the essential oil increases, efficacy increases and death occurs earlier.

Key words*: Drosophila suzukii (Matsumura), essential oils, Lavandula angustifolia, Rosmarinus oficinalis, Mentha piperita.*

INTRODUCTION

The repeated use of insecticides to control pests on crops leads to environmental pollution, disruption of the ecological balance, destruction of pollinators and beneficial entomofauna, and very often to the development of resistance. Therefore, alternative solutions are sought (Dam et al., 2019; Tait et al., 2021).

Plant insecticides have been used to control pests for centuries (Isman, 2006). The majority of plant products are biodegradable, therefore there is increasing interest in the use of plant extracts or essential oils for pest control (De Groot and Schmidt, 2016).

It is known that some chemical constituents of essential oils have insecticidal properties, their toxic effect is due to bioactive components such as mono- and sesquiterpenoids (Spitzer, 2004; Isman, 2020a; Chaudhari et al., 2021), which is why they can be used in both conventional and integrated plant protection.

Secondary compounds from plants, including alkaloids, terpenoids, phenols and flavonoids, can cause death of insects and act as attractants, detergents, phagostimulants, antifeedants or affect their reproductive processes (Smet et al., 1986; Houghton, 1996).

The effectiveness of essential oils depends on environmental conditions, photodegradation and evaporation (Pavela, 2014; Isman, 2020b).

Economic losses caused by *D. suzukii* reach $511 million per year in the United States alone, with additional costs associated with monitoring and control required to prevent attacks by this pest (Goodhue et al., 2011; Lee et al., 2011). Plant-based insecticides, essential oils and their compounds, can be used as alternative means to control *D. suzukii* (Isman and Grieneisen, 2014; Bernardi, 2017; Amoabeng et al., 2019; Souza et al., 2022; de Albuquerque et al., 2024; Dos Santos et al., 2024). They contain chemical compounds that cause death or delay the development of resistance (Isman, 2020b). Essential oils are toxic to *D. suzukii* larvae and adults (Jang et al., 2017). Furthermore, many essential oils disrupt the sense of smell of *D. suzukii* and act as repellents (Souza et al., 2022).

Rosemary (*Rosmarinus ofcinalis* L.) is one of the plants from which essential oils are extracted that have insecticidal activity. Chromatographic analyses of rosemary essential oil have shown

the presence of the following compounds: α-pinene, 1,8-cineole, borneol, bornyl acetate, camphor and verbenone (Isman et al., 2008; Sayorwan et al., 2013).
According to Renkema et al. (2016) most essential oils repel *D. suzukii*. Peppermint oil consists of two monoterpenoids menthol and menthone and is particularly effective. It acts as a repellent for up to 6 days.
Lavender oil contains the monoterpene components 1,8-cineole, carene and linalool, which act as fumigants for *D. suzukii* (Erland et al., 2015).
In this regard, essential oils have been tested for the control of *D. suzukii*.

MATERIALS AND METHODS

The studies were conducted under laboratory conditions (temperature 22-23°C, relative humidity 40-50% and photoperiod 16:8 - L:D). Essential oils of lavender, rosemary and mint were tested against adults of *Drosophila suzukii*. The experiment was set up in seven variants and three replicates: Variant I. Lavender - 0.1%, Variant II. Lavender - 0.2%, Variant III. Rosemary - 0.1%, Variant IV. Rosemary - 0.2%, Variant V. Mint - 0.1%, Variant VI. Mint - 0.2%, Variant VII. Control.
Blueberry fruits were placed in plastic containers, treated with the essential oils in the corresponding concentration. After that, 10 adults of *D. suzukii* were released into each container. Readings were made after 24 hours, on the 3rd and 5th days.
The efficiency is calculated using the Henderson-Tilton formula:

$$E\% = \left(1 - \frac{TaxCb}{TbxCa}\right).100$$

Ta - number of live in the variant after spraying;
Tb - number of live in the variant before spraying;
Ca - number of live in the control after spraying;
Cb - number of live in the control before spraying.
The processing of the obtained data was carried out with a package of statistical programs Statistika 7 V.

RESULTS AND DISCUSSIONS

In the first readings of the 24th hour, there are statistically proven differences between the efficacy of lavender 0.1% and mint 0.1% essential oils, with mint being higher (Figure 1). On the third day, there is only a statistically proven difference between rosemary 0.1%, lavender 0.1% and mint 0.1%. The highest efficacy is demonstrated by rosemary essential oil at a concentration of 0.1% (Figure 2).

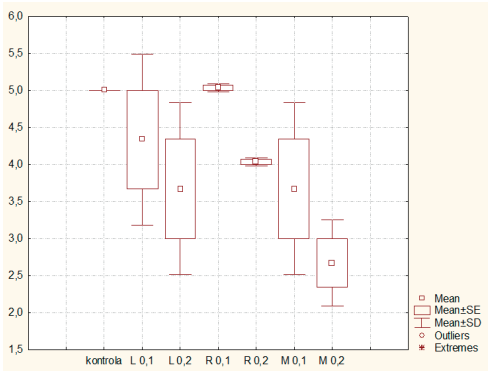


Figure 1. Comparing 24-hour averages

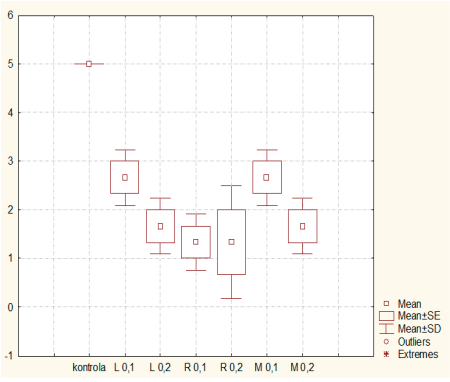


Figure 2. Comparison of average values on day 3

n the fifth day, there was a statistically significant difference between rosemary 0.2% and mint 0.1% (Figure 3).
The highest efficacy was demonstrated by rosemary at a concentration of 0.2%. These data are confirmed by studies by other authors Renkema et al. (2016).

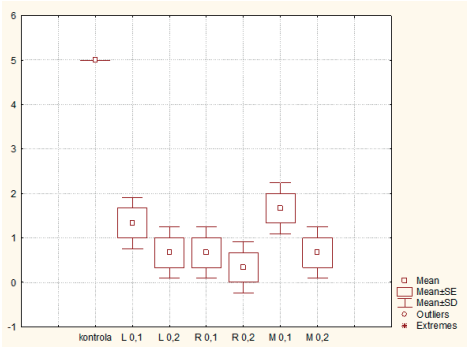


Figure 3. Comparison of average values on day 5

A regression analysis was conducted with the results of the research. Functional dependencies between the concentration of the working solution and the flies were obtained.

The obtained models are adequate at a significance level of p = 0.001.

In the case of lavender, the proposed model and graph show that the number of flies decreases at an essential oil concentration of 2.0 ml on the second day. At a lower amount of essential oil of 1.2 ml, the density of flies decreases on the 3rd day, and at a concentration of 1.0 ml, their number decreases only on the 5th day (Figure 4).

Table 1. Duration of action and amount of lavender oil in the working solution on *D.suzukii* adults

Regression Summary for Dependent Variable: z (lavender) R = ,89846037 R? = ,80723104 Adjusted R? = ,74297472 F(3,9) = 12,563 p						
	Beta	**Std. Err.**	**B**	**Std. Err.**	**t(9)**	**p-level**
x	1,31475	0,358487	0,471429	0,128542	3,66751	0,005175
y	1,29195	0,306116	1,155556	0,273799	4,22046	0,002238
xy	-1,96396	0,448108	-0,514286	0,117342	-4,38278	0,001764

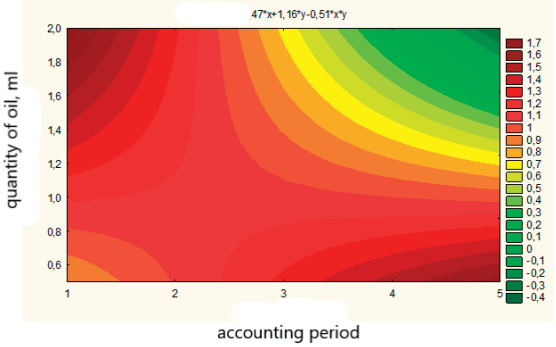


Figure 4. Flies counted after treatment with lavender oil

Table 2. Duration of action and amount of rosemary oil in the working solution on adults of *D.suzukii*

Regression Summary for Dependent Variable: z (rozmarin) R = ,94515842 R? = ,89332443 Adjusted R? = ,86961875 F(2,9) = 37,684 p						
	Beta	**Std. Err.**	**B**	**Std. Err.**	**t(9)**	**p-level**
Intercept			3,43229	0,254726	13,47446	0,000000
y	-0,734974	0,108871	-1,13333	0,167879	-6,75089	0,000084
x2	-0,594254	0,108871	-0,05134	0,009406	-5,45834	0,000401

When using rosemary oil, it is seen from the proposed model and graph that the number of flies decreases dramatically when the amount of oil in the solution is 2.0 ml on the second day (Figure 5). With a smaller amount of essential oil, the duration of action is extended. At 1.4 ml, the number of flies decreases on the 4th day, and at 1.0 ml after the 5th day. With increasing the concentration of the essential oil, the duration of the destruction of the enemy is reduced.

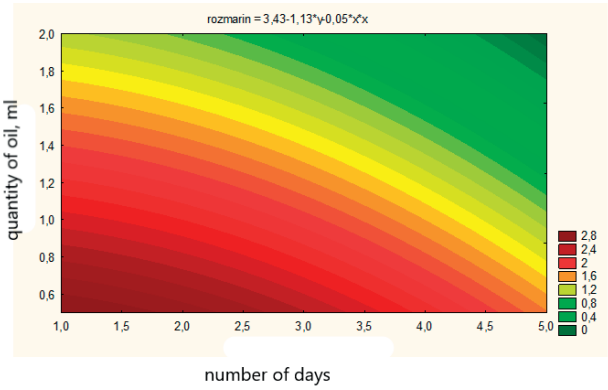


Figure 5. Flies counted after treatment with rosemary oil

Table 3. Duration of action and amount of peppermint oil in the working solution on *D.suzukii* adults

Regression Summary for Dependent Variable: z (menta) R = ,92867066 R? = ,86242920 Adjusted R? = ,81084015 F(3,8) = 16,717 p						
	Beta	**Std. Err.**	**B**	**Std. Err.**	**t(8)**	**p-level**
Intercept			6,80208	0,936704	7,26172	0,000087
y	-2,47850	0,744703	-5,56667	1,672591	-3,32817	0,010413
x2	-0,60312	0,131135	-0,07589	0,016501	-4,59922	0,001757
y2	1,88461	0,744703	1,66667	0,658582	2,53069	0,035218

With the mint from the proposed model, it is seen that at a concentration of the essential oil in the solution of 1.2-2 ml, the density of flies decreases after the fourth day (Figure 6).

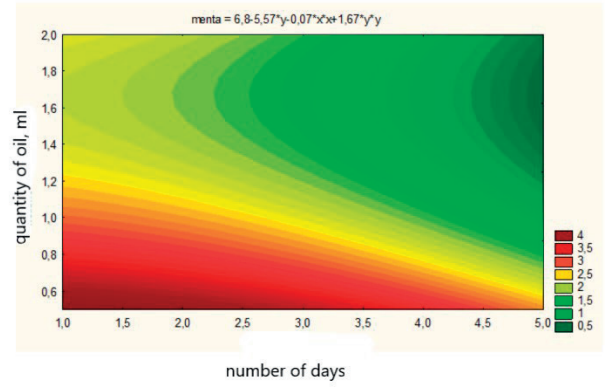


Figure 6. Flies counted after treatment with peppermint oil

According to Erland et al. (2015) lavender oil acts as a fumigant for *D. suzukii*, due to the presence of monoterpene components - 1,8-cineole, carene and linalool.

The toxic effect of peppermint oil is associated with the presence of menthol (monoterpene type), menthofuran, menthone, menthol esters - menthol acetate and valerate, phellandrene, pinene, cineole, menthofuran, piperitone, jasmone, tannins, flavonoids (luteolin, apigenin, diosmetin), as well as mustard, phenolic acids and mineral salts (İşcan et al., 2002; Kumar et al., 2011; Renkema et al., 2016). This oil also acts as a repellent.

According to Souza et al. (2022) rosemary oil shows better efficacy of 50% and 90% against adults compared to spinetoram. The compounds contained in rosemary essential oil - pinene, 1,8-cineole and camphor act as repellents against adults of *D. suzukii*. Females prefer to lay their eggs in untreated fruits compared to those treated with rosemary essential oil.

Botanical products are an alternative for controlling crop pests. They can be applied in organic farming and reduce the risk of developing resistance.

CONCLUSIONS

As a result of the conducted research, the following conclusions can be drawn:
- Against adult forms of *Drosophila suzukii* (Matsumura), the highest efficacy was reported for rosemary essential oil (*Rosmarinus ofcinalis* L.), followed by peppermint (*Mentha piperita* L.) and lavender (*Lavandula angustifolia* Mill.).
- With increasing concentration of essential oils, efficacy increases and death occurs in a shorter time.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

ASSESSMENT OF THE EFFECTS OF SLOW-RELEASE FERTILIZERS APPLICATION OVER THE AMOUNT OF NITROGEN LEACHED AND OF THE DEVELOPMENT OF WINTER WHEAT PLANTS IN CONTROLLED CLIMATE CONDITIONS

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Abstract

Slow-release fertilizers provide a steady supply of plant nutrients over an extended period of time. The objective of these investigations was to study what was the amount of nitrogen leached after the administration of nitrogen fertilizers and how it influenced the development of plants during the vegetation period. The experiment was carried out in pots of vegetation with winter wheat. The experiment was conducted in the green house with controlled climate conditions within ICAM Iasi - USV Iasi. This paper presents the results obtained regarding the influence of 5 nitrogen fertilizers, 2 classic fertilizers and 3 slow-release nitrogen fertilizers. The experiment was carried out in pots of vegetation with winter wheat. The amount of nitrogen leached after the administration of nitrogen fertilizers is influenced by the chemical forms of nitrogen included in the chemical composition of the fertilizers, but also by the structure, texture, chemistry of the soil and its fertility status. The amount of nitrogen leached is significantly lower for Sulfammo 25 and 30 variants compared to N2017TAR, Ammonium Nitrate, Ureea and the control, for both experiments.

Key words*: slow-release fertilizers, traditional fertilizer, nitrogen leached, soil chemistry.*

INTRODUCTION

The efficient use of nutrients by plants is highly dependent on the correct management of chemical fertilizers. The type of fertilizer applied can have a significant effect on crop yield (Stevens et al., 2005). Optimal fertilizer management must take into account the four pillars of fertilization: fertilizer type, optimal dose, timing of application, and the crop for which the fertilizer is intended (Volf & Raus, 2023).

Nitrogen-based fertilizers, although essential for crop development, exhibit a major drawback: more than three-quarters of their nutrient content is lost before being absorbed by plants. Urea and ammonium nitrate, the most frequently used nitrogen sources, rapidly degrade into ammonia - which evaporates into the atmosphere - and nitrates, which are highly susceptible to leaching. To compensate for these substantial nutrient losses, farmers are compelled to apply additional fertilizer quantities, consequently increasing production costs and reducing the overall economic efficiency of crops (Houlès et al., 2004; Laurent & Mary, 1992).

The extent of nitrogen loss following the application of nitrogen fertilizers depends on both the chemical form of nitrogen contained in the fertilizer and specific soil characteristics, including soil structure, texture, chemical properties, and overall fertility (Constantin et al., 2010; Addiscott, 2004).

Under typical conditions, nitrogen losses due to leaching can range between 10% and 30% of the total amount applied (Meisinger & Delgado, 2002). However, under adverse conditions - such as heavy rainfall, sandy soils, or excessive fertilization - nitrogen losses can rise significantly, reaching up to 50% (Baligar et al., 2001). On sandy soils, even under standard conditions, these losses are typically higher, ranging from 30% to 50%, and can further increase significantly with intensive irrigation or high applications of conventional nitrogen fertilizers associated with intensive agriculture (Beaudoin et al., 2005). Among the various nitrogen fertilizers, those containing nitrogen in nitrate form (NO_3^-) have the highest susceptibility to leaching compared to fertilizers based on ammonium (NH_4^+) or urea (Addiscott, 2004).

Through leaching, nitrates migrate from the soil into groundwater, thereby contaminating both soil and groundwater drinking sources. Nitrate concentrations often exceed the maximum permissible limit of 50 mg NO_3^-/L (Directive (EU) 2020/2184). The quantity of nitrate entering the soil via leaching is substantial, commonly ranging from 50 to 150 kg NO_3^-/ha/year or even higher (Beaudoin et al., 2005).

Selecting appropriate chemical fertilizers and ensuring correct application practices can substantially mitigate nitrogen losses through leaching, typically maintaining them below the 15-20% threshold. This approach considerably reduces environmental impacts and leads to significant economic savings (Constantin et al., 2010).

An increasingly adopted modern and effective strategy for reducing nitrogen losses and enhancing fertilizer efficiency is the use of slow-release or controlled-release fertilizers. The popularity and implementation of these advanced fertilizer formulations have grown markedly in recent years (Robbins, 2005).

Slow-release chemical fertilizers are formulated to gradually release nutrients into the soil over an extended period. This controlled release ensures a steady nutrient supply to plants throughout the growing season. As a result, nutrient concentrations in the soil solution remain at moderate levels, preventing excessive leaching of nitrogen and phosphorus into groundwater or surface water sources (Liu et al., 2020). This mechanism significantly reduces the risk of water pollution, particularly eutrophication, caused by fertilizer runoff. Additionally, ammonia volatilization and denitrification - the release of nitrogen gas from the soil - re lower compared to conventional readily available fertilizers, as high soil nitrogen concentrations do not accumulate over short periods.

The use of these fertilizers can reduce nitrogen losses through leaching by 30-50%. Under conditions such as sandy soils, high rainfall, or intensive irrigation, reductions in leaching losses can be even more substantial, reaching 40-60% (Trenkel, 2021; Guertal, 2009). Consequently, nutrient use efficiency, particularly nitrogen utilization, is significantly improved. Furthermore, by mitigating nitrogen losses through leaching or volatilization, the overall efficiency of nitrogen uptake from chemical fertilizers is enhanced (Morgan et al., 2009).

For farmers, the adoption of these fertilizers allows for a reduction in the number of fertilization applications per season. Since nutrients are released gradually over an extended period, certain intermediate fertilization steps can be eliminated. This reduction in applications translates into time savings, lower labor requirements, and decreased fuel consumption costs (Volf & Raus, 2023; Liu et al., 2014).

Research indicates that the use of controlled-release fertilizers can achieve crop yields comparable to those obtained with conventional fertilizers but with 20-30% lower total nitrogen application rates (Liu et al., 2020). As a result, farmers can decrease the amount of fertilizer applied while maintaining similar yields, leading to significant financial savings and a reduction in negative environmental impacts (Guertal, 2009; Morgan et al., 2009).

MATERIALS AND METHODS

Slow-release fertilizers primarily contain essential macronutrients - nitrogen (N), phosphorus (P), and potassium (K) - with nitrogen often receiving greater emphasis, typically in the form of urea or other nitrogen-based salts. In addition to N, P, and K, many slow-release formulations may also incorporate micronutrients such as iron, zinc, manganese, boron. However, the overall concentration of fertilizing elements in these products is generally lower compared to conventional concentrated fertilizers.

The basic chemical formula of the nutrients is similar to that of conventional fertilizers, including compounds such as urea, ammonium phosphates, sulphates or potassium chlorides. The main difference lies in the mechanism controlling solubilization. Various technologies have been developed in the fertilizer industry, leading to the classification of fertilizers according to their mechanism of action. Thus, there are slow-release fertilizers, controlled-release fertilizers and mixtures of active substances with different solubilization rates. The difference between these categories is

determined by how the active substance is released into the soil.

The primary objective of this study was to conduct a comparative analysis of the amount of nitrogen leached following fertilizer application and its influence on plant development throughout the growing season. To achieve this, five types of fertilizers with distinct characteristics were tested, considering factors such as chemical composition, the forms of nitrogen present in their formulations, solubilization degree, nutrient release mechanism in the soil, and effectiveness in minimizing leaching losses (Table 1).

Table 1. Characterization of tested fertilizers

Fertilizers	Solubility in water at 20°C (g/L)	Chemical composition	Specifications	Dissolution rate
T1 Sulfammo 25	600	25% Nt 18% NH_4 7% NO_3 2% MgO 31% SO_3	MPPA DUO - Activated Poly-Phenolic Molecules XCK: Plant extract	Medium
T 2 Sulfammo 30	550	30% Nt 5% NH_4 25% Urea 3% MgO 15% SO_3 0.15% B 0.10% Zn	Organocalcium matrix Microbial activator N-PRO, enzyme activator	Low
T3 N2017 TAR	n/a	n/a	unspecified	to be determined
T4 AN 34 (ammonium nitrate)	1180	34% Nt 17% NH_4 17% NO_3	-	Very fast
T5 Urea 46	1080	46% Nt 46% $CO(NH_2)_2$	-	Fast

The experiment was conducted in a climate-controlled greenhouse at ICAM - USV Iași, as well as in temperature-regulated climate chambers, enabling precise monitoring of experimental parameters, including soil characteristics, humidity, temperature, and light intensity. Detailed information regarding the experimental parameters is provided in Table 2. The experiment was performed using vegetation pots, with fall wheat as the test species. Constant-volume vegetation pots were filled with two soil types exhibiting distinct chemical properties (Table 3). The experimental design followed a randomized block design with four replications. Each vegetation pot was considered a single replicate, from which four soil and plant samples were collected and subsequently analyzed. The primary chemical characteristic used in selecting the tested soil types was soil pH.

Table 2. Experimental parameters

Conditions	Parameters
Vegetation pots Pot diameter Soil column height Soil bulk density Pot surface Soil weight	 20 cm 20 cm 1.1 g/cm^3 314 cm^2 7 kg/pot
Cultivated species	*Triticum aestivum* L., Ariesan variety
Sowing density / pot / ha	 18 seeds/pot 500 no of seeds/m^2
Fertilizer application N doses (equivalent/ha) T1 – Sulfammo 25 T2 – Sulfammo 30 T3 – N2017TAR T4 – AN 34 T5 – Urea 46	dissolved in 150 ml of distilled water 25 kg a.s. N/ha 0.38 g/pot 0.31 g/pot 0.39 g/pot 0.28 g/pot 0.21 g/pot
Low temperature simulation -1.1 / -2.9°C	Climatic chamber 5 days
Precipitation simulation Distilled water equivalent/m^2	BBCH 29 0.630 l/pot 20 l/m^2
Chlorophyll content – initial values	BBCH 10-12
Chlorophyll content - stage I	BBCH 25-26
Chlorophyll content - stage II	BBCH 35-37
Total Nitrogen leachate - collector	BBCH 29-30
Total Nitrogen in plants	BBCH 30-32

The physicochemical analysis of the soil samples was conducted in accordance with the ICPA Bucharest methodology, which serves as the standard protocol in all agrochemical laboratories across Romania. The following parameters were assessed: soil pH, total nitrogen, accessible phosphorus, accessible potassium, total calcium carbonate, and particle size distribution. To determine leached nitrogen, water samples were collected from the designated collectors. To determine the total nitrogen content in water samples, the STAS 7184/2-85 standard method was applied. This method involves a wet oxidation process using concentrated sulfuric acid and a catalyst to digest nitrogen-containing compounds into ammonium, followed by a colorimetric determination using Nessler's reagent or phenol-hypochlorite reaction, depending on the detection system. The absorbance is measured spectrophotometrically, and nitrogen concentration is calculated based on a calibration curve.

Plant growth analysis included the determination of total nitrogen content and protein content

using the Kjeldahl method according to SR ISO 3188:1995. The method involves acid digestion of plant material with sulfuric acid and a catalyst, followed by distillation and titration to quantify the nitrogen content, which was then converted to protein using a standard factor.

Chlorophyll content was assessed using a Chlorophyll Content Meter (CCM-200 plus, Opti-Sciences, USA), which estimates relative chlorophyll concentration non-destructively by measuring light transmittance at two wavelengths (red and near-infrared) through the leaf tissue. Stomatal conductance was measured with a SC-1 Leaf Porometer (Decagon Devices, USA), which determines the rate of water vapor diffusion through the stomata, providing an indirect indicator of plant water status and gas exchange capacity.

Statistical analysis was performed using SPSS software, applying analysis of variance (ANOVA). Differences between variants were statistically evaluated using Duncan's test ($p < 0.05$).

RESULTS AND DISCUSSIONS

Soil characterization in vegetation pots

It is well established that soil reaction (pH) significantly influences both the fixation and availability of nitrogen from fertilizers, exerting a major impact on biological and chemical processes within the soil.

The retention of nitrogen forms from fertilizers varies depending on soil pH. Ammoniacal nitrogen (NH_4^+) is more effectively fixed in acidic or neutral soils (pH 5.5–7), where it is adsorbed onto clay and humus particles. In contrast, in alkaline soils (pH >7.5), NH_4^+ is converted into gaseous NH_3^+, leading to volatilization losses. Nitric nitrogen (NO_3^-) remains highly mobile and is poorly retained in soils regardless of pH, making it particularly susceptible to leaching in acidic and permeable soils, especially at pH levels below 5.5. Amide nitrogen (urea, $CO(NH_2)_2$) undergoes rapid hydrolysis to NH_4^+; however, at pH levels above 7, this process is accompanied by the volatilization of gaseous ammonia (NH_3) (Volf & Raus, 2023).

Based on these considerations, experiments were conducted on two soil types with distinct chemical properties, the compositions of which are detailed in Table 3.

In this context, two experiments were designed, maintaining all factors constant except for soil type and its corresponding pH. The first experiment utilized an alkaline soil (pH = 8.38), characterized by high phosphorus and potassium content, moderate nitrogen levels, and a fine silt texture. The second experiment employed a neutral soil (pH = 7.04), which was well-supplied with potassium, had moderate nitrogen and phosphorus levels, and exhibited a texture closely resembling that of the soil used in the first experiment (Table 3).

Table 3. Physico-chemical soil characterization

Soil analysis	Units	Soil 1		Soli 2	
		Mean values	Characteristics	Mean values	Characteristics
pH	-	8.38	Moderate alkalinity	7.04	Neutral
Total Nitrogen	%	0.193	Medium	0.149	Medium
Available Phosphorus	ppm	78	Very high	30	Medium
Accessible Potassium	ppm	498	Very high	331	Very high
Total calcium carbonate	%	4.16		0.63	
Soil texture			Medium texture		Medium texture
Coarse sand (2.0-0.2 mm)	%	1.0	Fine silt	0.8	Fine silt
Fine sand (0.2-0.02 mm)	%	31.2		34.5	
Silt (0.02-0.002 mm)	%	38.1		29.3	
Clay (< 0.002 mm)	%	29.5		35.4	
Macroscopic clay (< 0.01 mm)	%	49.9		52.4	

Leached Total Nitrogen (LTN)

The amount of nitrogen leached from nitrogen fertilizer applications is influenced by both the chemical forms of nitrogen present in the fertilizer composition and soil properties, including structure, texture, chemical composition, and fertility status.

The quantity of leached nitrogen was significantly lower for Sulfammo 25 and Sulfammo 30 compared to N2017TAR, AN 34, Urea 46,

and the control in both trials. Ammoniacal nitrogen (NH_4^+) was more efficiently retained in neutral soil (pH = 7.04), where it was adsorbed onto clay and humus particles. In contrast, unprotected urea was rapidly solubilized, leading to a significant loss of total nitrogen (Table 4).

Due to the chemical composition of Sulfammo 25 and Sulfammo 30, as well as the presence of ammonium ions (NH_4^+), which have a high adsorption capacity on the colloidal complex and low mobility within the soil, the total nitrogen content in the leached water was lower compared to the other treatments. This can be attributed to the fact that the other fertilizer variants contained nitrogen in the form of nitrate (NO_3^-), which, due to its negative ionic charge and high mobility, is more prone to leaching. In alkaline soil, nitrogen losses in the form of nitrate were more pronounced, resulting in higher levels of total leached nitrogen (Table 4). The greater amount of leached nitrogen in the N2017TAR and Urea 46 treatments can also be explained by the high content of amide nitrogen (N-amide), which is susceptible to leaching through the soil profile due to its low polarization. Additionally, the conversion of N-amide in the soil occurred rapidly due to the high temperatures recorded during the test period, which accelerated its transformation into nitric nitrogen (N-nitric). Other factors, such as soil texture, structure, and organic matter content, also influenced the rate of nitrogen leaching.

Table 4. Leached Total Nitrogen (LTN, % DM)

Treatments	LTN (%) Alkaline Soil pH	LTN (%) Neutral Soil pH
T1 Sulfammo 25%	0.256[a]	0.398[a]
T2 Sulfammo 30%	0.455[a]	0.512[a]
T3 N2017TAR	0.796[b]	0.568[a]
T4 AN 34%	0.810[b]	0.682[ab]
T5 Urea 46%	0.824[b]	0.896[b]
T6 Unfertilized	0.106[c]	0.114[c]

[1]Values followed by the same letter in the exponent are not significantly different at $p \leq 0.05$ (Duncan test)
[2]DM = Dry Matter
[3]DM = 88.0%

Total Nitrogen (TN) and Crude Protein (CP) Content in Plants

The total nitrogen (TN) content in the dry matter was higher for Sulfammo 25, Sulfammo 30, and N2017TAR compared to the other fertilizers. This can be attributed to the greater amount of solubilized nitrogen remaining in plant-available forms (Table 5).

The differences in total nitrogen content between the two trials were influenced by variations in the pH of the nutrient medium. It is well established that the uptake intensity of NH_4^+ increases with pH, with neutral pH conditions favoring its absorption.

In alkaline soils, the total nitrogen (TN) content, expressed as a percentage of dry matter, falls within a very low safety class according to the ICPA scale, which may lead to nitrogen deficiency across all fertilization treatments. Conversely, in neutral pH soils, the TN content was 3.63% for the Sulfammo 25 treatment (classified as low assurance) and 5.86% for the Sulfammo 30 treatment (classified as normal assurance) (Table 5) These findings suggest that, when Sulfammo 30 was applied, the ammoniacal nitrogen contained in the fertilizer remained available in the soil for a longer period, was translocated more efficiently within the plant, and was optimally metabolized.

Table 5. Total Nitrogen (TN) and Crude Protein (CP) Content in Plants Expressed on a Dry Matter Basis (% DM)

Treatments	TN (%) Alkaline Soil pH	CP (%) Alkaline Soil pH	TN (%) Neutral Soil pH	CP (%) Neutral Soil pH
T1 Sulfammo 25%	2.09[a]	13.06[a]	3.63[ab]	22.69[a]
T2 Sulfammo 30%	2.21[a]	13.81[a]	5.86[a]	36.63[a]
T3 N2017TAR	1.93[b]	12.06[a]	4.90[a]	30.63[a]
T4 AN 34%	1.13[b]	7.06[b]	3.45[ab]	27.81[a]
T5 Urea 46%	1.36[b]	8.50[b]	2.16[b]	13.50[b]
T6 Unfertilized	1.20[b]	4.75[b]	1.63[c]	11.69[b]

[1]Values followed by the same letter in the exponent are not significantly different at $p \leq 0.05$ (Duncan test)
[2]DM = Dry Matter
[3]DM = 88.0%
[4]The results for TN and CT are expressed on a DM basis.

Effect of Different Treatments on Stomatal Conductance (SC)

Stomatal conductance (SC) was measured to assess plant water use efficiency and its impact on photosynthesis. Chemical fertilizers, particularly nitrogen-based fertilizers, play a crucial role in plant metabolism by influencing soil water and nutrient uptake, enhancing resistance to external stresses, promoting leaf mass development, and ultimately affecting yield. However, no significant differences were observed between fertilization treatments.

Soil pH influences stomatal conductance (SC) through multiple mechanisms, affecting nutrient availability, plant hormone balance, and root system health. All tested fertilizers resulted in higher stomatal conductance values in neutral

pH soils compared to alkaline pH soils (Table 6). An optimal pH (typically between 6 and 7) supports efficient stomatal function, maximizing gas exchange and photosynthesis. In contrast, alkaline soil conditions can induce physiological stress in plants by stimulating the synthesis of abscisic acid (ABA), which triggers stomatal closure to reduce water loss.

Table 6. Effect of Different Treatments on Stomatal Conductance (SC, mmol m^{-2} s^{-1}) under Alkaline and Neutral Soil pH Conditions

Treatments	SC (mmol m^{-2} s^{-1}) Alkaline Soil pH	SC (mmol m^{-2} s^{-1}) Neutral Soil pH
T1 Sulfammo 25%	31.2[a]	68.8[a]
T2 Sulfammo 30%	31.3[a]	68.0[a]
T3 N2017TAR	29.4[a]	64.9[a]
T4 AN 34%	31.2[a]	49.0[a]
T5 Urea 46%	31.6[a]	55.6[a]
T6 Unfertilized	49.7[b]	68.8[a]

[1]Values followed by the same letter in the exponent are not significantly different at $p \leq 0.05$ (Duncan test)

Effect of Different Treatments on Chlorophyll Content (CCI)

Soil pH influences plant chlorophyll content by regulating the availability of essential nutrients involved in chlorophyll synthesis and photosynthetic processes. An imbalanced pH can lead to mineral deficiencies, negatively affecting both the quantity and stability of chlorophyll in leaves.

The dynamics of chlorophyll content exhibited distinct trends in the two experiments due to differences in the pH of the nutrient medium. An alkaline pH environment facilitated the uptake of NH_4^+ from fertilizers with a high nitrogen content in this form (Table 7).

Table 7. Effect of Different Treatments on Chlorophyll Content (Chlorophyll Content Index, CCI units) under Alkaline Soil pH Conditions

Treatments	CCI – BBCH 10-12	CCI – BBCH 25-26	CCI – BBCH 35-37
T1 Sulfammo 25%	5.0[a]	9.4[a]	12.5[b]
T2 Sulfammo 30%	5.3[a]	11.0[ab]	15.3[b]
T3 N2017TAR	4.3[a]	11.7[ab]	11.1[ab]
T4 AN 34%	4.2[a]	13.9[b]	9.9[ab]
T5 Urea 46%	5.1[a]	14.2[b]	4.3[a]
T6 Unfertilized	4.7[a]	9.8[b]	8.7[ab]

[1]Values followed by the same letter in the exponent are not significantly different at $p \leq 0.05$ (Duncan test)
[2]Number of determinations – 15 (weighted average: base/middle/top – 5 plants – first leaf)

The form of nitrogen present in fertilizers significantly influences plant uptake, metabolism, and nitrogen content, directly affecting crop growth, photosynthesis, and yield. A comparison of the experimental variants revealed that, during the early stages of the growing season, chlorophyll content was lower in the Sulfammo 25, Sulfammo 30, and N2017TAR treatments compared to AN 34 and Urea 46, despite the higher total nitrogen content in the plants. As the growing season progressed, chlorophyll content increased in the Sulfammo 25, Sulfammo 30, and N2017TAR treatments, surpassing the values recorded for AN 34 and Urea 46.

This phenomenon can be attributed to the slower uptake of NH_4^+, which remained available to plants over an extended period. The reduced uptake rate of this nitrogen form facilitated a more balanced development between leaves and roots, promoted a higher protein content, and consequently led to a later increase in chlorophyll content. While Urea 46 provided a balance between the effects of NH_4^+ and NO_3^-, it was also associated with high nitrogen losses (Table 8).

Table 8. Effect of Different Treatments on Chlorophyll Content (Chlorophyll Content Index, CCI units) Under Neutral Soil pH Conditions

Treatments	CCI – BBCH 10-12	CCI – BBCH 25-26	CCI – BBCH 35-37
T1 Sulfammo 25%	1.2[a]	3.3[bc]	3.3[a]
T2 Sulfammo 30%	1.2[a]	2.6[abc]	3.1[a]
T3 N2017TAR	2.0[a]	2.5[ab]	3.6[a]
T4 AN 34%	1.8[a]	3.4[c]	1.8[b]
T5 Urea 46%	2.1[a]	3.2[bc]	2.1[b]
T6 Unfertilized	1.9[a]	2.0[a]	-

[1]Values followed by the same letter in the exponent are not significantly different at $p \leq 0.05$ (Duncan test)
[2]Number of determinations – 15 (weighted average: base/middle/top – 5 plants – first leaf)

Relationship Between Leached Total Nitrogen and Plant Total Nitrogen Content

An analysis of the relationship between leached total nitrogen (LTN, %) and total plant nitrogen content (TN, %) reveals that the fertilizers Sulfammo 25 and Sulfammo 30 exhibit the lowest LTN values (Figure 1). This suggests a higher nitrogen retention efficiency in the soil, thereby reducing nitrogen losses through leaching. Concurrently, these fertilizers contribute to the highest total nitrogen accumulation in plants, ensuring a sustained nitrogen supply throughout the growing season. Their effectiveness is particularly notable in moderately alkaline soils.

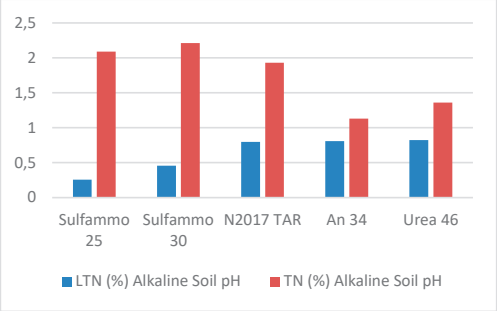


Figure 1. Relationship Between Leached Total Nitrogen and Plant Total Nitrogen Content in Alkaline Soil

Conversely, AN 34 and Urea 46 displayed the highest nitrogen leaching losses (LTN, %), indicating their potential suitability for soils with a high nutrient retention capacity or for application in controlled fertilization systems (Figure 2). Consequently, under the experimental conditions analyzed, the total nitrogen content in plants (TN, %) was significantly lower when these fertilizers were applied.

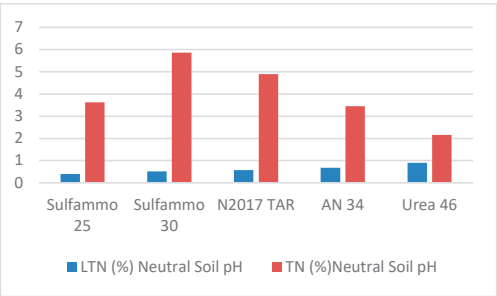


Figure 2. Relationship Between Leached Total Nitrogen and Plant Total Nitrogen Content in Neutral Soil

The analysis of data from neutral pH soils confirms the same ranking of fertilizer effectiveness as observed in alkaline pH soils. Sulfammo 30 and Sulfammo 25 emerged as the most effective fertilization options, demonstrating superior nitrogen uptake and minimized leaching losses. In contrast, AN 34 and N2017 TAR exhibited moderate total plant nitrogen content (TN, %). However, AN 34 experienced higher nitrogen leaching losses, which may contribute to reduced agronomic efficiency.

Comparison of Chlorophyll Content and Total Plant Protein Content

Correlation analysis between crude protein content and chlorophyll content in alkaline-reacting soils does not indicate a directly proportional relationship between these variables (Figure 3). However, Sulfammo 30 recorded the highest crude protein percentage (14%), suggesting a more efficient accumulation of nitrogen in plant proteins. In contrast, AN 34 exhibited the lowest crude protein content (6%), indicating a reduced conversion of nitrogen into plant protein. Although the variants fertilized with AN 34 and Urea 46 showed the highest chlorophyll content values, their crude protein levels remained low. This finding suggests that while AN 34 and Urea 46 enhance chlorophyll synthesis, they do not significantly contribute to protein accumulation. Consequently, the nitrogen supplied by these fertilizers appears to be preferentially utilized for photosynthetic metabolism rather than for protein biosynthesis.

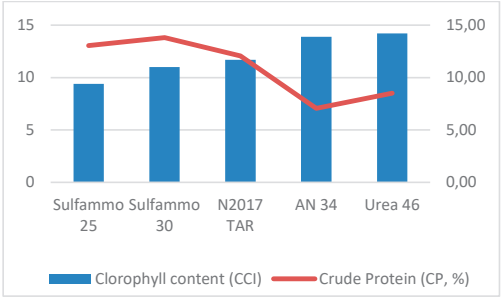


Figure 3. Relationship Between Chlorophyll Content and Total Plant Protein Content in Alkaline Soil

In neutral-reacting soils, AN 34 exhibited the highest chlorophyll content, followed by Urea 46 and N2017 TAR, suggesting that these fertilizers enhance the efficiency of photosynthetic processes (Figure 4).

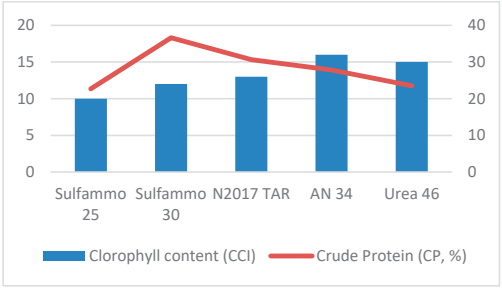


Figure 4. Comparison of Chlorophyll Content and Total Plant Protein Content in Neutral Soil

Conversely, Sulfammo 30 recorded the highest crude protein content (CP %) but did not achieve

the highest chlorophyll levels. This finding indicates that the nitrogen supplied by this fertilizer is utilized more efficiently for protein synthesis rather than for the production of photosynthetic pigments. N2017 TAR displayed intermediate values for both chlorophyll and crude protein content, suggesting a balanced allocation of nitrogen between these two physiological processes.

CONCLUSIONS

Chlorophyll and Total Protein Content: Relationship and Fertilizer Influence

The absence of a direct correlation between chlorophyll content and total protein content suggests that nitrogen utilization varies depending on the fertilizer type and the physiological requirements of the plant.

AN 34 and Urea 46 primarily stimulate chlorophyll synthesis but do not significantly contribute to protein accumulation. This observation indicates that the nitrogen supplied by these fertilizers is predominantly used for primary plant metabolism, including photosynthetic processes, rather than for protein biosynthesis. This phenomenon can be attributed to the rapid uptake of nitrate nitrogen (NO_3^-), which accumulates in young plants, enhancing chlorophyll production but requiring additional metabolic conversion for protein synthesis.

Sulfammo 25 is particularly suited for alkaline soils or soils prone to high nitrogen leaching due to its slower nitrogen release. While it is more efficient in promoting crude protein accumulation than Urea 46 and AN 34, it does not reach the performance level of Sulfammo 30. Despite not stimulating chlorophyll synthesis to the same extent, Sulfammo 25 ensures an optimal balance between protein content and nitrogen use efficiency.

Among the fertilizers analyzed, Sulfammo 30 is the most effective in enhancing crude protein content, irrespective of soil pH. It achieves the highest CP (%) values, even though it does not result in the highest chlorophyll accumulation. The prolonged availability of ammonium nitrogen (NH_4^+) allows plants to utilize nitrogen efficiently, facilitating metabolic processes involved in protein biosynthesis. However, the overall efficiency of this fertilizer is influenced by factors such as crop type, soil properties, and microbiological activity, which regulate nitrogen transformations in the soil.

Total Plant Nitrogen and Total Leached Nitrogen

Soils with a neutral pH facilitate nitrogen uptake, regardless of the type of fertilizer applied. The total plant nitrogen content (TN, %) was significantly higher in neutral soils compared to alkaline soils across all treatments. The most substantial improvements were observed for Sulfammo 30 and Sulfammo 25, indicating that these fertilizers are particularly effective under neutral soil conditions. Their high efficiency can be attributed to their ammoniacal nitrogen (NH_4^+) content, which remains more stable in neutral soils and is less prone to losses through volatilization or leaching.

Intermediate but satisfactory TN (%) values were also recorded for N2017 TAR, which demonstrated greater effectiveness in neutral soils. This could be due to its composition, which likely includes both ammonium nitrogen (NH_4^+) and nitrate nitrogen (NO_3^-), providing greater adaptability in soils with variable pH. The improved nitrogen uptake in neutral soils is explained by the optimized nitrogen transformation processes and enhanced plant absorption.

Nitrogen Leaching and Fertilizer Susceptibility

Nitrogen leaching did not vary significantly between alkaline and neutral soils. However, the highest leaching losses were recorded for AN 34 and Urea 46, demonstrating their susceptibility to nitrogen loss, regardless of soil pH. These fertilizers contain nitrogen primarily in nitrate form (NO_3^-) or in forms that rapidly convert to NO_3^-, such as the rapid solubilization of urea, making them highly soluble and prone to leaching.

To minimize nitrogen losses, Sulfammo 25 and Sulfammo 30 are the most suitable fertilizers for both neutral and alkaline soils. These fertilizers contain nitrogen in ammoniacal (NH_4^+) and amide ($CO(NH_2)_2$) forms, which are more stable in the soil and less prone to leaching.

Nitrogen Stabilization Technologies

To enhance nitrogen retention and reduce losses due to leaching or fixation, Sulfammo 25 and Sulfammo 30 incorporate advanced stabilization technologies, including:

MPPA DUO - Activated Poly-Phenolic Molecules (Sulfammo 25)
Organocalcium Matrix (Sulfammo 30)
These components provide superior nitrogen protection (as detailed in Table 1). Active polyphenols in MPPA DUO slow the conversion of NH_4^+ to NO_3^- by partially inhibiting nitrification, thereby improving nitrogen retention in the soil and enhancing plant uptake. The Organocalcium Matrix stabilizes nitrogen through a combination of calcium and organic compounds, reducing leaching losses. Additionally, calcium influences soil pH and microbiological activity, thereby regulating the conversion rate of NH_4^+ to NO_3^-.

Recommendation

On both soil types (neutral and alkaline), Sulfammo 25 and Sulfammo 30 exhibited the lowest nitrogen leaching losses, confirming their status as the most effective fertilizers for minimizing nitrogen loss. Moreover, neutral soils significantly enhance plant nitrogen uptake, with Sulfammo 30 emerging as the best-performing fertilizer under these conditions.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

EFFICACY OF BIOSTIMULANTS AGAINST CABBAGE STEM FLEA BEETLES IN WINTER OILSEED RAPE UNDER FIELD CONDITIONS IN THE UK

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Abstract

This field study evaluated the efficacy of Yokosan and Converta biostimulants in mitigating cabbage stem flea beetle damage on winter oilseed rape (cv. Acacia). Treatments included control (no biostimulants) and varying doses of Yokosan (0.5, 1, and 2 L/ha) and Converta (1, 2, and 4 L/ha), applied at cotyledon, 6-leaf, and 8-leaf stages. The randomised complete block design included four replicates, with biostimulants applied at 300 L/ha per spray. Both biostimulants reduced beetle damage significantly compared to controls, which showed severe damage (60–70% of new growth affected). Yokosan was particularly effective at 1 L/ha, while Converta at 4 L/ha minimised leaf infestation (6.95 leaves/plant) and stem infestation (8%). Yokosan performed best at 0.5 L/ha for reducing stem larvae, though higher rates offered no further improvement. Conversely, Converta displayed dose-dependent efficacy for reducing beetle activity and infestation. Results suggest Yokosan and Converta can enhance plant health and resistance, with Converta at 4 L/ha emerging as the optimal treatment. These findings emphasise the importance of dose optimisation for integrated pest management strategies.

Key words*: biostimulants, Yokosan, Converta, cabbage stem flea beetles, winter oilseed rape, pest management, dose rates.*

INTRODUCTION

Oilseed rape (*Brassica napus*) is a key crop in the UK. It is grown for its oil, animal feed, and as a break crop to reduce pathogen and pest in cereal dominated rotations (Nicolls, 2016). Globally, it is the third-largest source of vegetable oil (Hegewald et al., 2018) and the second-largest source of protein meal (AHDB, 2023). In the UK and much of Europe, oilseed rape ranks as the third most widely grown crop after wheat and barley, and the fourth most productive arable crop following wheat, barley and oats (Defra, 2024).

A major threat to oilseed rape cultivation is the cabbage stem flea beetle (CSFB, *Psylliodes chrysocephala*), a stem mining pest, which attacks the crop during its establishment, significantly reducing yield and quality. Losses can exceed 20%, valued at over £120 million annually. Narrow profit margins have led to a more than 60% decline in UK oilseed rape cultivation in 2020 (Withall et al., 2024). It can also damage other brassica crops such as turnip, mustard and cabbage (Ahuja et al., 2011).

Adult CSFB feed on young OSR plants in autumn, while larvae mine within the petioles and stems until pupation in spring. Heavy feeding by adults can kill young plants if pest pressure is high. Controlling the pest is increasingly difficult due to limited effective treatments. The EU ban on neonicotinoid seed treatments in 2013 exacerbated the issue, further compounded by widespread resistance to pyrethroids, the only remaining conventional insecticides used for its control (Willis et al., 2020; Hoarau et al., 2022; Rüde et al., 2025).

Given these challenges, sustainable and environmentally friendly pest control alternatives are urgently needed. Many farmers have abandoned oilseed rape due to persistent pest pressure, but improved crop management practices could help mitigate damage. Companion planting, for example, has been shown to reduce cabbage stem flea beetle damage (Admin, 2024). Another promising approach is the use of plant biostimulants, which could offer an eco-friendly alternative to

synthetic pesticides (Rouphael and Colla, 2020).

Biostimulants contain a wide range of products used in agriculture to increase productivity, nutrient use efficiency, and improve plant tolerance to biotic and abiotic stresses (Bulgari et al., 2015). They can contain natural substances from plants and animals, as well as microorganisms. Due to their complex nature, often consisting of multiple molecules, identifying the specific active components responsible for their benefits on crops remain challenging (Yakhin et al., 2017). Moreover, their effects are not always consistent among the plant species (Bartwal et al., 2015). Their efficacy primarily depends on careful management before, during, and after application, depending on the biostimulant type. Biostimulants can, however, offer a promising alternative to agrochemicals, which can harm the environment and pose significant health risks to both humans and animals (Mojumdar et al., 2022).

To explore potential cabbage stem flea beetle control strategies, two biostimulants, Converta and Yokosan, were investigated for their ability to enhance oilseed rape resilience under field conditions. Converta is a specialised biofertiliser enriched with nitrogen-fixing, phosphorus-solubilizing, and potassium-mobilising bacteria. It unlocks the nutrients present in the soil, reducing the need chemical fertilisers. It has been developed to enhance plant nitrogen uptake, boost beneficial microbial activity and improve soil fertility to support plant growth. Yokosan is an organic liquid biofertiliser formulated with potassium humate, amino acid, and seaweed extract. It is enriched with beneficial microorganisms and a blend of macro- and micronutrients to promote plant health and productivity. The study compared biostimulant oilseed rape treated plants with an untreated control group to assess their effectiveness in managing cabbage stem flea beetle in oilseed rape.

MATERIALS AND METHODS

The study assessed the effectiveness of Yokosan and Converta biostimulants in managing the cabbage stem flea beetle on winter oilseed crop (cv. Acacia) under field conditions planted in August 2023. Yokosan was applied at 0.5, 1 and 2 L/ha, while Converta was applied at 1, 2 and 4 L/ha. Each rate of Yokosan and Converta biostimulants was applied at cotyledon, six leaf, and eight leaf stage. All applications were applied at a spray volume of 300 L/ha per application with the knapsack sprayer. The control plots received no biostimulants.

The trial was set up in a randomised complete block design with four replications. Each plot was 3 m wide and 20 m long. Assessments included beetle damage on new growth, number of leaves infested, and larval infestation in stems and leaf petioles. Beetle damage on new growth was assessed by observing biting spots on at least 10 randomly selected plants per plot 7 days after an application. In mid-February, 10 plants per plot were randomly collected and their leaf petioles and stems were dissected to count larvae using a magnifying glass. Data were analysed using one-way analysis of variance (ANOA) in Minitab. Tukey's test was applied to separate any treatment differences when ANOVA was found to be significant at the 5% probability level.

RESULTS AND DISCUSSIONS

Two biostimulants, Converta and Yokosan, were investigated as potential solutions to enhance plant resilience against cabbage stem flea beetles. The key findings are discussed below.

Cabbage stem beetle (csfb) damage on new growth

The percentage of new growth damaged by cabbage stem flea beetles following the biostimulant application at 6 and 8 leaf stages is presented in Figure 1.

Both Converta and Yokosan biostimulants, applied at different rates, significantly reduced beetle damage on new growth compared to the control. In the control group, 60-70% of new growth showed beetle damage. The damage decreased with higher biostimulant application rates, with Yokosan proving more effective than Converta. The lowest damage was noted in Yokosan plots treated at 1 L/ha. While Converta also reduced damage, its effect was slightly less pronounced at higher rates.

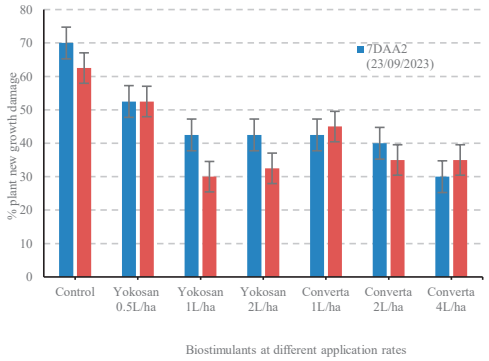


Figure 1. The effect of biostimulants applied at different rates at 6 leaf stage and 8-leaf stage on new growth of the oilseed rape under field conditions, planted in August 2023 (P = 0.017 & 0.036 at 6 leaf stage and 8 leaf stage, respectively). Control plots received no biostimulants. Three applications of each rate of Yokosan and Converta biostimulants were made at cotyledon stage, 6 leaf stage and 8 leaf stage, with a spray volume of 300 L/ha per application. New growth damage was recorded 7 days after application at both leaf stages. Error bars represent the standard error of means (n = 10)

The reduction in beetle activity may be attributed to better overall plant health, induced systemic resistance, increased nutrient uptake or a combination of all, making the plants less palatable or more resistant to beetle attack. Additionally, the biostimulants may have altered cell sap composition or taste, reducing plants less susceptible to beetle attack. More likely, the potassium-mobilising bacteria in Converta following application, may have enhanced potassium availability and uptake, consequently improving the plants' ability to withstand or deter beetle attack. This could also involve the production of secondary metabolites that acted as deterrents.

Plant secondary metabolites play a crucial role in plant-environment interactions and defence. They contribute to resilience against biotic and abiotic stresses by acting as phytoalexins, signal molecules and antioxidants (Bartwal et al., 2013). Additionally, biostimulants may have facilitated enhanced nutrient uptake and utilisation (Yakhin et al., 2017; Bhupenchandra et al., 2020), thus leading to stronger and more resilient plants that are less susceptible to damage.

Plant leaves infested with beetle larvae

The average number of plant leaves infested with cabbage stem flea beetle larvae by mid-February are summarised in Figure 2. Although statistically non-significant (P = 0.263), all biostimulant treatments reduced larval infestation compared to the control, indicating a potential positive impact on pest management. The 1 L/ha treatment had the highest larval infestation rate among the Yokosan treatments, with an average of 9.05 infested leaves per plant. The 0.5 L/ha and 2 L/ha treatments showed slightly lower infestation levels, at 8.30 and 8.72 infested leaves per plant, respectively. There was, however, no clear trend suggesting that higher rates of Yokosan significantly reduced leaf infestation.

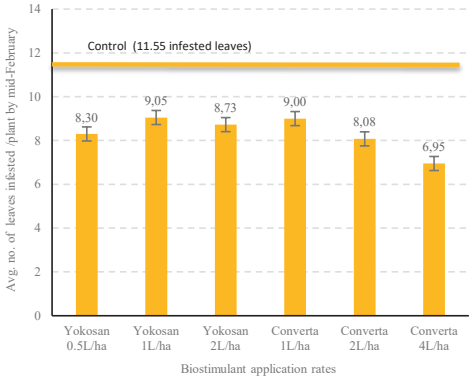


Figure 2. The effect of biostimulants applied at different rates on the number of OSR plant leaves infested with cabbage stem flea beetles' larvae by mid-February, planted in August 2023 (P = 0.263). Control plots received no biostimulants. Three applications of each rate of Yokosan and Converta biostimulants were made at cotyledon stage, 6 leaf stage and 8 leaf stage, with a spray volume of 300 L/ha per application. Error bars represent the standard error means (n = 10)

In contrast, Converta showed a decreasing trend in the number of infested leaves with increasing rates. The 4 L/ha Converta treatment appeared to be the most effective rate, with only 6.94 infested leaves per plant, followed by the 2 L/ha treatment, with 8.07 infested leaves per plant. The 1 L/ha treatment had an average of 9 infested leaves per plant, which is comparable to the control group.

Overall, the results indicate that higher biostimulants rates generally led to lower leaf infestation levels, with Converta at 4 L/ha being the most effective treatment, indicating a dose-dependent effect. The higher rate might

have increased nutrient availability and uptake (Bhupenchandra et al., 2020), promoting healthier plants and enhancing resilience against adult beetles and larvae during the establishment phase. Additionally, the increased rate might have triggered physiological defence mechanisms in the treated plants, such as changes in sap composition and taste, making them less palatable to adult beetles and subsequently reducing larval infestation.

Beetle larvae in petioles and stems in mid-February

A summary of the mean number of cabbage stem flea beetle larvae found in the petiole and stem in mid-February is shown in Figure 3.

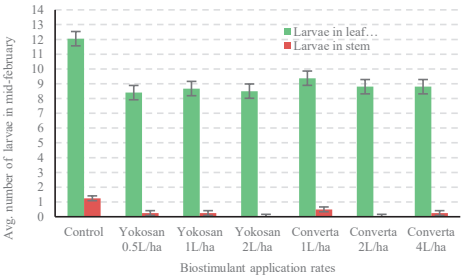


Figure 3. The effect of biostimulants applied at different rates on the number of cabbage stem flea beetle larvae found in the leaf petiole (P = 0.945) and stems (P = 0.247) of oilseed rape in mid-February, planted in August 2023. Control plots received no biostimulants. Three applications of each rate of Yokosan and Converta biostimulants were made at cotyledon stage, 6 leaf stage and 8 leaf stage, with a spray volume of 300 L/ha per application. Error bars represent the standard error of the means (n =10)

There were no significant differences between treatments for larvae in petioles (P = 0.945) and stems (P = 0.247). On average, both biostimulants, Yokosan and Converta, were effective in reducing the cabbage stem flea beetle larvae numbers compared to the untreated control. Among the two, Yokosan appeared to be more effective in reducing larvae. Interestingly, the lowest application rate of Yokosan (0.5 L/ha) was more effective than higher rates. This suggests that there might be an optimal dose rate for Yokosan where it is most effective and increasing the rate beyond this would not further significantly enhance its effectiveness against the larvae. It is also postulated that Yokosan treated plants might have triggered a strong immune response, thus reducing larval infestation in leaf petioles and subsequently in the stems. For Converta, there was a slight trend indicating that higher application rates may be more effective, but the differences were not noticeable. This might imply that beyond a certain point, increasing the rate may not substantially improve Converta's effectiveness in reducing larvae. Additionally, the results suggest that Converta treated plants, regardless of application rate, may have undergone physiological changes that enhanced their resilience to larval attack. Since newly hatched larvae feed on the stems and lower petioles over winter and into spring (Willaims, 2004), these changes may have contributed to reduced petiole and stem infestation during the establishment phase.

CONCLUSIONS

This study indicates that biostimulants have the potential to enhance plant resilience and reduce cabbage stem flea beetle damage in oilseed rape. Yokosan was more effective overall, with optimal rate at 0.5 L/ha, while Converta showed a dose dependent reduction in infestation, with 4 L/ha being the most effective. Further research is, however, needed to validate their effectiveness and optimise application strategies, potentially providing a sustainable pest management solution for oilseed rape growers.

ACKNOWLEDGEMENTS

The author is thankful to Russell Bio Solutions UK for supplying the biostimulants used in this field study.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

MODELING BIOLOGICAL GROWTH OF *Lolium perenne* SPECIES UNDER CURRENT CLIMATE CHANGE CONDITIONS

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Abstract

The importance of knowing the impact of life factors is also supported by several specialized studies. Several measurements were made on 34 local biotopes collected from Almajului Mountains, Mehedinți Plateau and Blahniței Plain. During the vegetation period, recording was investigated: plant height, leaf dimensions, and leaf surface of the shoot, along five BBCH development codes. Depending on the bio-accumulations of essential life factors (sum of accumulated precipitation and temperatures), biological growth was estimated. The mathematical interpretation of the slopes of the lines represents the growth rate, which practically determines the influence of precipitation or temperatures on the studied characters. The results of the research in this work show that the determining factor in the dynamics of biological growth in perennial ryegrass is the amount of precipitation. Perennial ryegrass growth in the presence of precipitation is almost double that of the accumulation of an identical amount of temperature. Moreover, studies published in related research reveal that the major limiting factors are drought and high temperatures, factors that are accentuated by current climate change.

Key words*: perennial ryegrass, life factors, biological growth.*

INTRODUCTION

Previous studies have shown that current climate change will improve forage production conditions, as the growing season will be longer (1-3 months), with milder and rainier autumns and winters (Sampoux et al., 2011; Cui et al., 2018; Zhangab et al., 2019). It is demonstrated that in the last decade the climate has warmed by 1-2^{0}C, under these conditions the dominant plurispecific plant associations of perennial ryegrass will constitute one of the most valuable fodder resources (Nakamura et al., 2008; Blackmore et al., 2016; McDonagh et al., 2016; Giraldo et al., 2018).

The drought tolerance of this species is low, this fact limits the area of distribution especially in the current context of global climate change (Yates et al., 2019; Hatier et al., 2014; Westermeier et al., 2016).

In other words, the current global climate change characterized by expected seasonal changes in precipitation periods associated with increased frequencies of temporary drought periods will affect the production capacity of perennial ryegrass (Westermeier & Hartmann, 2016). The response to water stress in perennial ryegrass depends on the interactions between factors that ensure intraspecific community diversity such as soil properties, climatic conditions, and management practices (Buttler et al., 2019; De Swaef et al., 2021).

Drought causes a decrease in productivity, but the availability of certain productive characters can mitigate drought resistance, such as leaf size, bush diameter, plant height, leaf:stem ratio, root system architecture (Hofer et al., 2016; Lee et al., 2019).

Water and nutrients, especially nitrogen, are major limiting factors in forage crop species, influencing their efficiency in growth and development and, therefore, reducing biomass production. Limiting water availability in perennial ryegrass has the effect of reducing the availability of carbon assimilated from photosynthesis contributes to vegetative development through stomatal conductance. Under these conditions, limiting the amount of CO_2 in leaves slows down the respiration rate but also the water loss per unit area (Akmal & Janssens, 2004; Wagner et al., 2008).

According to numerous studies, the survival of perennial ryegrass under drought conditions has been associated with a series of traits: root length and mass, photosynthetic characteristics and water-soluble carbohydrate concentrations. It has been proven that water stress in this species decreases the photosynthetic capacity due to the reduction of the active leaf area, decreasing its efficiency depending on the rate of onset and extension of the stress caused by water shortage. Drought is an important environmental stress factor in agriculture worldwide, due to climate change by destabilizing production, affecting both yield and quality (Pearson et al., 2011; Jonavičienė et al., 2014; Kemesyte et al., 2017).

Temperature is also one of the limiting factors for the growth cycles of perennial ryegrass, throughout the entire vegetation period from planting to harvest (Calvache et al., 2021; Wei et al., 2023).

The low temperature resistance of the species is a complex trait meaning the ability to survive and grow at low temperatures, to acclimatize to cold, to tolerate wind, snow cover, etc. (Skøt et al., 2002; Höglind et al., 2011). Perennial ryegrass tolerates poorly drained soils, with a pH range between 5.0 and 8.3 and optimal growth occurs between 20 and 25^0C.

The plant's ability to adapt to extreme living conditions includes the ability to survive and grow at low temperatures, to acclimatize to cold, to tolerate wind and snow, etc. High temperatures limit performance in warmer and transitional regions (Schmidt et al., 2015; Skøt et al., 2002). Also, perennial ryegrass is a forage species that has a pronounced response to temperature and light and does not flower unless exposed to long periods of cold followed by long days with high temperatures (Ciannamea et al., 2007; Heineck et al., 2020). The flowering period of ryegrass is an important trait, as it determines the reproductive power of the plant because during evolution the species has adapted mechanisms to synchronize flowering and seed setting with favorable environmental conditions. Prolonged exposure to low temperatures, long/short photoperiods promote or repress flowering (Bergonzi & Albani, 2011; Butnariu & Cauni, 2013). In light of these considerations, we generally consider that heat stress is a major abiotic stress that affects the productivity and growth of forage plants by disrupting metabolic homeostasis and the activities of various biochemical processes, such as photosynthesis, respiration, and stress defense (Lei et al., 2022; Kemesyte et al., 1017). Knowledge of the dynamics of productive characters such as leaf size, bush diameter, plant height, leaf: stem ratio is important because it influences the regeneration capacity after defoliation and allows the restoration of active photosynthesis and plant growth. As a result of the externalization of these characters, perennial ryegrass becomes one of the dominant species in temperate grasslands, ensuring a rapid establishment for this species, increased tolerance to defoliation, but also a production with high digestibility.

It is known that the productive traits of perennial ryegrass are influenced especially in the vegetative stage by factors such as the height of the vegetation cover, the frequency of defoliation or N fertilization (Meuriot et al., 2018). The frequency of defoliation influences the recovery of the species, moreover, frequent defoliation reduces the persistence and quality of the forage obtained from the harvest of perennial ryegrass (Calvache et al., 2020; Terán-Chaves et al., 2022).

These elements are important because monitoring the spatio-temporal dynamics of current climate change on the quality and quantity of aboveground biomass produced is important and contributes to adjusting the management decision-making process, such as the density of the vegetation cover, mowing time or fertilizer application rates (Pranga et al., 2021; Toporan, 2024).

MATERIALS AND METHODS

Biological material

The biological material was represented by 34 biotypes collected from 3 areas of differentiated altitudinal range: Almajului Mountains, Mehedinți Plateau and Blahniței Plain. These biotypes were collected from natural grassland habitats and then transferred to the germplasm collection field at the Lovrin Agricultural Research and Development Station, Timis, Romania, on an identical agro-background for all biotypes. The codes BBCH 31, BBCH 37, BBCH 55, BBCH 71, and BBCH 89 were

mainly investigated, which correspond to the vegetative and generative growth stage.

The soil was a typical chernozem soil, considered representative of the Low Banat Plain area. The pH value indicated a weakly alkaline reaction in the range of 20-100 cm, to even strongly alkaline at depths of up to 200 cm (Bostan et al., 2024).

The purpose of the study

The purpose of the study was focused on the biological growth depending on the amounts of precipitation and temperatures accumulated by the plant during the vegetation period. For that the amounts of precipitation and daily temperatures were recorded, using descriptively their sum at each BBCH. With the help of this information, the mathematical quantification of the biological growth response of the main productive characters was represented: plant height, leaf dimensions, and leaf surface of the shoot.

Climatic conditions

The analysis of data on the rainfall regime in the period 2023-2024 registered at Lovrin Agricultural Research and Development Station, shows that it was an atypical year overall. The total amount of precipitation was 417.4 mm, with a negative deviation of 104 mm from the multiannual average. During the perennial ryegrass vegetation period, the amount of precipitation was 121.6 mm, with a negative deviation of 134.6 mm from the MMA.

The average annual temperature was 14.6^0C, 3.6^0C higher than the 70-year multiannual average. During the perennial ryegrass vegetation period, temperatures exceeded the multiannual average monthly. The largest deviations from the average were reported starting with February and until July. Thus, in February a positive deviation of $+7^0$C was recorded, in March + 4.3^0C, in April $+3.1^0$C, in June $+3.8^0$C, and in July $+4.6^0$C.

During the vegetation the perennial ryegrass biotopes accumulated the following climatic factors: the amount of precipitation was between 62.5 and 196.4 mm, and the sum of temperatures between 454.1 and 1061.8^0C. Since the increases at 1^0C, or 1 mm of precipitation are subunits, it is considered appropriate to express the dynamics of the studied characters at average accumulations of 10 ^{0}C or 10 mm of precipitation.

Statistical processing

The perennial ryegrass growth biological development was estimated, for this, both the experimental data and the regression lines associated with them were represented in the same coordinate system. The mathematical interpretation of the slopes of the lines represents the growth rate, which practically determines the influence of precipitation or temperatures on the studied characters.

To test the presence of a significant link between the biological growth of the selection biotope and the main growth factors, one-way ANOVA was also used.

The graphical representations were made with JASP version 0.18.3 (Goss-Sampson, 2018; Langsrud, 2003; Whitlock & Schluter, 2015).

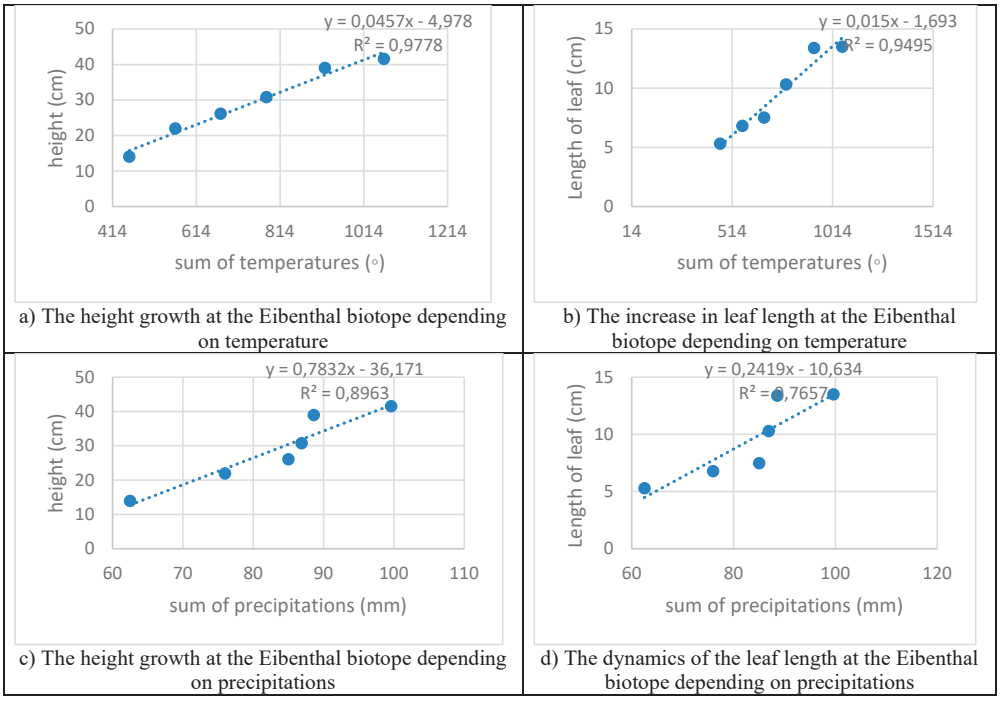
RESULTS AND DISCUSSIONS

To cope with current climate change, it is necessary to create new cultivars adapted to current abiotic conditions. From the analysis of climate conditions throughout 2024 with a non-uniform median distribution throughout the biological growth period, was performed the growth measurements in five BBCH development codes.

Biological growth of selected biotopes from the Montane area - Almaj Mountains

The altitudinal range of the collection was between 86 m and 740 meters. The orography is diverse, which primarily produces an edaphic differentiation, not identifying a common soil type for the five localities from where the biological material was collected.

The analysis of the growth dynamics of the biotopes collected from this area reveals a maximum increase in height at the accumulation of 10^0C in the case of the Eibenthal and Mraconia with 0.45 cm. At these biotopes, a response towards the maximum was also recorded, quantified with an increase of 0.79 cm at the accumulation of ten mm of precipitation.



a) The height growth at the Eibenthal biotope depending on temperature

b) The increase in leaf length at the Eibenthal biotope depending on temperature

c) The height growth at the Eibenthal biotope depending on precipitations

d) The dynamics of the leaf length at the Eibenthal biotope depending on precipitations

Figure 1. The graphical representation of biological growth at Eibenthal at accumulation depending on temperature and precipitation (a, b, c, d)

Biological growth of selected elites from the Mehedinți Plateau Area

This area is characterized by an altitudinal range between 104 m and 654 meters, the dominant soil type is the typical districambosol soil. The analysis of the biological response to temperature accumulation is differentiated, the maximum increase in height was at Gornovița and Ponoarele biotopes where it is associated with an increase in leaf length of 0.079 cm at an accumulation of 10^0C (Figure 2).

The influence of precipitation on the dynamics of height growth was extremely differentiated. The maximum increases exceeding 0.9 cm are recorded at the Gornovița and Turbata biotopes followed by the Ponoarele with 0.84 cm.

Biological growth of selected elites from the Câmpia Blahniței Area

This collection area had an altitudinal range between 49 m and 120 meters. The analysis of the biological response to temperature accumulation distinguishes the Cioroboreni and Burila Mare biotopes with an increase between 0.48-0.56 cm at the accumulation of 10^0C.

The influence of precipitation on the growth dynamics revealed that at the accumulation of 10 mm of precipitations the maximum increases was recorded at the same biotopes Cioroboreni at 0.96 cm, and Burila Mare at 0.82 cm (Figure 3).

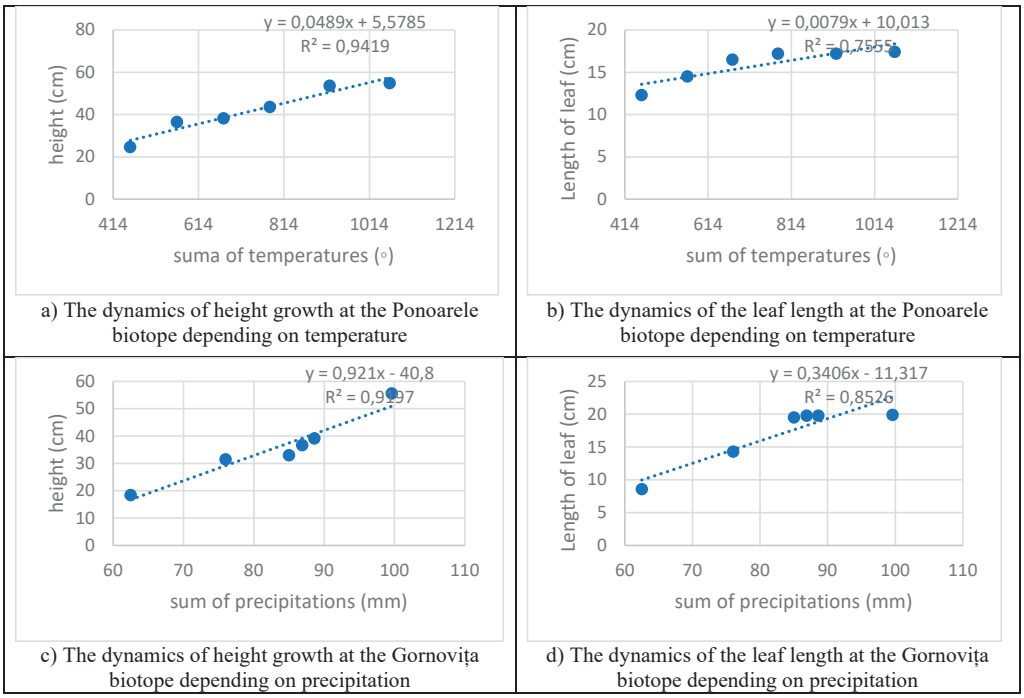


Figure 2. The graphical representation of biological growth at Ponoarele (a, b) and Gornovița (c, d) biotopes depending on temperature and precipitation

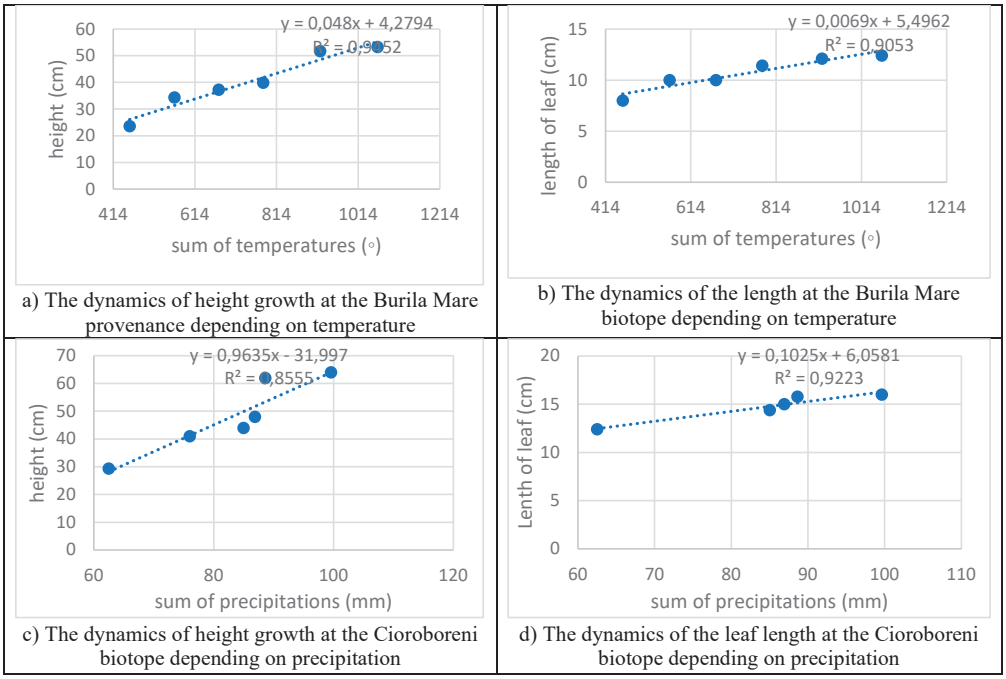


Figure 3. The graphical representation of biological growth at Burula Mare (a, b) and Cioroboreni (c, d) biotopes depending on temperature and precipitation

Analysis of biological growth according to abiotic factors using the ANOVA test

Analysis of variance ANOVA was used as a statistical tool to analyze the means obtained between the groups of experimental data obtained. Thus, in this research, we analyzed the means obtained from the biological growth of short-crop ryegrass in order to establish the presence or absence of statistically significant interdependencies or differences between it and the major growth factors. We chose this type of test because it has the capacity to test two groups of experimental data to identify the connection between their means. Table 1 presents the one-way ANOVA analysis of variance on the biological growth of perennial ryegrass in height at the accumulation of 10^0C. In this case, the variability was due to the analysis of the differences between the data groups, namely the growth in height of perennial ryegrass at the accumulation of 10^0C.

In order to analyze the significance level of the P value, we compared the F value determined using the critical values from the previous distribution table. Thus, in this case the significance value of P is 0.60, which does not reject the null hypothesis and does not statistically assure us of the existence of a significant interdependence between the group means.

Table 1. One-way ANOVA on height growth at 10^0C

Groups	Count	Sum	Average	Variance		
Almajului Mountains	5	1.97	0.39	0.013		
Mehedinți Plateau	18	6.18	0.34	0.011		
Blahniței Plain	11	4.09	0.37	0.011		
Source of Variat.	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.012	2	0.00	0.515	0.602	3.304
Within Groups	0.37	31	0.01			
Total	0.38	33				

ns p>0.05; * p≤0.05; ** p≤0.01; *** p≤0.001

In order to analyze the significance level of the P value, we compared the F value determined using the critical values from the previous distribution table. Thus, in this case the significance value of P is 0.60, which does not reject the null hypothesis and does not statistically assure us of the existence of a significant interdependence between the group means. As is known from specialized studies, there is a close connection between the persistence of perennial ryegrass and climatic factors. Thus, normal development of the foliar apparatus is considered to occur at 20^0C (Calvache et al., 2020).

Table 2 presents the one-way ANOVA analysis of variance on the height increase of local perennial ryegrass provenances in height upon accumulation of 10 mm of precipitation. Thus, in this case the significance value of P is 0.13 which does not reject the null hypothesis and does not statistically assure us of the existence of a significant interdependence between the group means.

Table 2. One-way ANOVA analysis of variance on height growth at 10 mm of rainfall

Groups	Count	Sum	Average	Variance		
Almajului Mountains	5	4.09	0.81	0.011		
Mehedinți Plateau	18	11.24	0.62	0.04		
Blahniței Plain	11	7.7	0.7	0.02		
Source of Variat.	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.15	2	0.07	2.14	0.13	3.30
Within Groups	1.11	31	0.03			
Total	1.26	33				

ns p>0.05; * p≤0.05; ** p≤0.01; *** p≤0.001

CONCLUSIONS

It is scientifically proven that this species has an excellent capacity to control the expansion and optimize light interception by increasing the proportion of leaf surface, if the availability of nutrients in the soil is improved (Lakić & Vojin, 2015; Deru, et al., 2014).

The results obtained regarding the biological growth of the 34 local perennial ryegrass provenances revealed a better adaptation of the biological material from the Blahniței Plain area compared to that collected from the Plateau or Mountain area.

The ANOVA analysis of variance revealed that the relationship between the biological development of perennial ryegrass and major abiotic factors has the best significance when comparing the data groups between leaf blade length and the accumulation of 10^0C in the plant. The significance value of P was 0.040, this value rejects the null hypothesis and statistically assures us of the existence of a significant interdependence between the dynamics of leaf dimensions and the accumulation of temperature.

The results of this research show that the determining factor in the dynamics of biological growth is the amount of precipitation. The growth of perennial ryegrass in the presence of precipitation is almost double compared to the accumulation of an identical amount of temperature. Moreover, studies published in related research reveal that the major limiting factors are drought and high temperatures, factors accentuated by current climate change (Förster et al., 2018).

It is expected that in the future, global climate change will cause decreases in agricultural production due to lack of water during the growing season. To overcome this problem, a genetic improvement of drought tolerance is needed by creating and expanding new varieties in cultivation. Consequently, improving the adaptability of perennial forage plants to high and/or low temperature can increase forage yield and turf quality in regions where their biological development is strongly affected (Zhao et al., 2016; Förster et al., 2018).

ACKNOWLEDGEMENTS

The support of this study was carried out within the project with the theme: "The role of biomass in environmental conservation, rural development and the resilience of agricultural farms", carried out by the University of Life Sciences "King Mihai I" from Timisoara, Romania"

Also, we would like to express our gratitude to the Department of Forage Plant Breeding from the Lovrin Agricultural Research Station for the support provided in conducting the experiments and processing the results.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

STUDY OF THE EFFECT OF SOME FOLIAR HERBICIDES ON ALFALFA (*Medicago sativa* L.) IN CHANGING CLIMATE CONDITIONS

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Abstract

In a study, conducted during the period 2023-24, it was found that weed control in alfalfa is difficult to achieve with a single herbicide application. The efficacy varies depending on the species composition of the weeds. The highest efficacy against Eastern groundsel, Prickly lettuce and Shepherd`s purse was reported when Corum - 125 ml/da is applied, against Field pennycress and Flixweed with Pulsar Plus - 160 ml/da, against Milk thistle with Cleranda SC - 200 ml/da. The highest phytotoxicity was registrated at variant treated with Onix - 100 ml/da - score 4, and it is not completely overcome and remains visible until the first swath. At all other other variants, the phytotoxicity is weaker and is overcome by the plants. The highest chlorophyll index was recorded with untreated control, and the lowest with Onyx treatment – 100 ml/da at the first reporting, with the values of the indicator increasing at subsequent reading dates. Foliar herbicides treatments does not unidirectionally affect the plants height, as well as the yield of green and dry mass.

Key words*: alfalfa, weeds, weed control, yield.*

INTRODUCTION

According to Radović et al. (2009) alfalfa is a forage crop of great importance. It is having a protein-rich forage with high digestibility. The sown areas with alfalfa in Bulgaria for 2023 were 85 536 ha, and the total dry mass production - 279 873 tons (https://www.mzh.government.bg)

Managing the weeds in the stands is a critical part of alfalfa growing (Beck et al., 2017). In alfalfa fields a great number of weeds are developing together with the crop: Annual - *Amaranthus retroflexus, Chenopodium album, Stellaria media, Lamiun aplexiculare, Sinapis arvensis, Anthemis arvensis, Capsella bursa-pastoris, Polygonum convolvulus, Erygeron canadense, Echinochloa crus-galli,* etc.; Perennial - *Convolvulus arvensis, Cirsium arvense, Sonchus arvensis, Cynodon dactylon, Sorghum halepense, Rumex crispus*, etc. A big problem is the parasitic weed *Cuscuta* spp. (Bogatsevska et al., 2008; Tonev et al., 2019). The weed management in alfalfa have to be performed by integrated approach combining preventive, cultural and chemical means (Bogatsevska et al., 2008; Tonev et al., 2019).

A great number of studies are being conducted worldwide related to chemical weed control in alfalfa. Herbicides with high crop selectivity have been registered for weed control in alfalfa (Srinivasan et al., 2006; Zhang et al., 2010). According to Dillehay et al. (2010) and Meiss et al. (2010), the realization of the full biological potential of alfalfa and the longevity of the crop are closely related to the competitive impact of weeds depending on the degree and duration of weeding.

Both soil and foliar herbicides have been found to be highly effective against most annual monocotyledonous and dicotyledonous weed species that infest alfalfa stands (Dimitrova, 2001; Arregui et al., 2001; Cummings et al., 2004; Dimitrova, 2007; Meighani et al., 2010). Devine et al. (1993) found that plant species and varieties exhibit different sensitivity to a particular herbicide. Similar studies in Bulgaria are limited. Marinov-Serafimov and Kertikova (2011) found specific varietal responses of alfalfa genotypes to the herbicidal product Pledge 50 VP.

The critical period for weed control has been well studied in many crops, and the results are used to develop recommendations for better weed management. However, the research on the critical period for weed control in alfalfa is limited. With the advent of new, more effective foliar herbicides, establishing the critical period may further improve yields. The objective of a study conducted by Dillehay et al. (2011) was to

determine the critical period for weed control in spring-sown alfalfa. In 2004, the critical period varied depending on location, but began at the 0.5 tri-foliate growth stage of alfalfa and ended at the 7 tri-foliate growth stage.

The herbicides metribuzin and simazine applied in the fall were evaluated in field trials conducted from 1978 to 1980 in western Nebraska for selective weed control in non-irrigated, established alfalfa. All herbicides effectively controlled the weed Bromus tectorum. Broadleaf weeds were controlled to varying levels by the herbicides tested. All herbicide treatments increased yield and protein content compared with the untreated control (Wilson, 1981).

The efficacy of spring-applied herbicides was evaluated in western Nebraska from 1982 to 1984 for selective weed control in alfalfa. Weed densities were lowest in plots treated pre-plant with benefin in combination with post emergence applications of 2,4-D. Combinations of sethoxydim with bromoxynil and fluazifop-butyl with bromoxynil provided lower control of the grass weeds than sethoxydim or fluazifop-butyl applied alone (Wilson, 1986).

Marinov-Serafimov et al. (2016) conducted an experiment in 2014-2015 at the Institute of Forage Crops – Pleven, Bulgaria, studying the selectivity of three herbicides Listego 40, Cleranda, and Stratos ultra at alfalfa, variety Dara. The digestibility of dry and organic matter of the forage in all variants of the experiment varied in the range from 59.61 to 60.88%, and did not depend on the selectivity of the studied herbicides to alfalfa.

There is still low amount of information for the influence of different herbicides to alfalfa. Therefore, the aim of this study is to establish the efficacy of various herbicide products on against some weeds in alfalfa and the influence of treatments on the growth and reproductive performance of the crop.

MATERIALS AND METHODS

The experiment was carried out in the experimental field at the experimental field of the Agricultural University – Plovdiv, Bulgaria. The experiment was carried out in two consecutive years - 2023 (3rd vegetation of alfalfa) and 2024 (4th vegetation of alfalfa). The experiment was set up using the long plots method. The plot size is 60 m².

The experiment included the following variants: 1. Untreated control; 2. Cleranda SC (375 g/l metazachlor + 17.5 g/l imazamox) – 2.00 L ha^{-1}; 3. Pulsar 40 (40 g/l imazamox) – 1.20 L ha^{-1}; 4. Pulsar Plus (25 g/l imazamox) – 1.60 L ha^{-1}; 5. Corum (480 g/l bentazone + 22.4 g/l imazamox) – 1.25 L ha^{-1}; 6. Onix 60 EC (600 g/l pyridate) – 1.00 L ha^{-1}. The herbicides were applied in the phenophase 3rd – 5th fully developed tri-foliate of alfalfa (BBCH 13-15).

For the purpose of the experiment, an alfalfa variety was grown - "PR 54 Q 14" from Corteva Agriscience (https://www.corteva.bg). The sowing was performed in the autumn of 2022 after preceding crop winter wheat.

Weed infestation in alfalfa is represented by: *Senecio vernalis* Waldst. & Kit. – 14 pieces/m^2 in 2023 and 18 pieces/m^2 in 2024; *Lactuca serriola* L. – 10 pieces/m^2 in 2023 and 15 pieces/m^2 in 2024; *Capsella bursa-pastoris* L. – 7 pieces/m^2 in 2023 and 10 pieces/m^2 in 2024; *Thlaspi arvense* L. – 5 pieces/m^2 in 2023 and 7 pieces/m^2 in 2024; *Sonchus oleraceus* L. – 9 pieces/m^2 in 2023 and 5 pieces/m^2 in 2024; *Descurainia sophia* L. – 6 pieces/m^2 in 2023 and 8 pieces/m^2 in 2024.

The efficacy of the herbicides was assessed using the 10-score visual scale of EWRS (European Weed Research Society) on the 14th and 28th days after treatments. The selectivity of the herbicides was assessed using the 9-score EWRS scale on the 7th and 14th days after treatment (1. No symptoms; 2. Very weak symptoms - weak suppression; 3. Weak but easily recognizable symptoms; 4. More pronounced symptoms (e.g. chlorosis); 5. Thinning, severe chlorosis or suppression, with expected yield reduction; 6. Severely damaged to complete death /Moderately bad/; 7. Severely damaged to complete death /Poor/; 8. Severely damaged to complete death /Very bad/; 9. Severely damaged to complete death /Absolutely bad/).

The following indicators were recorded for alfalfa: - Leaf chlorophyll content index (CCI Index) - 7, 14 and 21 days after treatment (only at the first swath). The measurement was performed with a portable chlorophyll measuring device (CCM-200 plus Chlorophyll Content Meter) from ADC BioScientific Ltd.,

UK (https://www.optisci.com/ccm-200.html). The chlorophyll content index was recorded on twenty randomly chosen alfalfa plants from a variant, and the average values from the twenty measurements are presented.

- Plant height at the first and second swath (cm). Measurements were made on thirty plants of each variant;
- Fresh and dry mass at the first and second swath (t ha^{-1}). To perform statistical analysis, each plot was divided into three parts of 20 m^2 to obtain three replications. Each 20 m^2 was mowed separately and the alfalfa was weighed in a fresh state. To determine the dry mass, the alfalfa mass from each 20 m^2 of the plot was spread out and periodically turned over for drying and weighed again separately. The results were averaged per plot and converted into t ha^{-1}. The first swath was done in the budding stage of the alfalfa. The second swath was done 35 days after the first.

For statistical processing of the data, the Duncan method was used with the SPSS 19 program. Differences were considered significant at $p<0.05$.

RESULTS AND DISCUSSIONS

On table 1 the average amount of precipitation, as well as the average minimum and maximum air temperatures during the alfalfa growing seasons (for the period January-December in 2023 and for the period January-August in 2024). According to the meteorological data, we can determine how agro-climatic conditions affect the development of alfalfa.

Table 1. Average monthly precipitation (mm) and average monthly minimum and maximum air temperatures (C°) during the trial period (2023/2024)

Months	Precipitation		min t°	max t°	min t°	max t°
	2023	2024	2023		2024	
January	36.8	47.8	1.6	11.2	-1.9	9.9
February	21.3	6.8	-0.5	14.2	2.6	17.4
March	14.8	39.3	2.3	17.3	4.9	17.7
April	70.3	37.3	6.4	20.3	8.4	25.8
May	78.8	74.5	10.8	23.5	11.2	24.3
June	60.8	5.3	14.8	31.3	17.5	35.1
July	31.8	11.3	18.7	37.8	18.9	37.1
August	21.4	19.5	17.8	36.7	17.9	36.8
September	15.0	-	14.4	30.9	-	-
October	2.3	-	8.6	26.5	-	-
November	52.8	-	3.9	15.8	-	-
December	51.0	-	-0.2	12.1	-	-

It is seen from Table 1 that the amount of precipitation during the months from March to June in the two experimental years (2023 and 2024) contributed to relatively good moisture storage and the production of two swaths.

The temperatures (min and max) were suitable for the plants development. Despite the high winter temperatures, no negative impact of the warm winter months on the growth and development of alfalfa was found. There were higher temperatures in the second experimental year, especially in the period June-August.

During the experimental period, no more than two swaths of alfalfa were made due to the low amounts of precipitation during the period July-September.

Based on the analysis of meteorological data, we can indicate the two experimental years as relatively unfavorable for the growth, development and realization of the productive potential of alfalfa.

The efficacy of the studied herbicides is presented in 6 tables. Similar efficacy was found during the two experimental years, with efficacy being lower on the first reporting date and higher on the second.

On Table 2 is presented the efficacy against *S. vernalis*. On average for the period, on the 14th and 28th day after treatments, the highest efficacy was recorded after the application of Corum – 1.25 L ha^{-1} – 75 and 92.5% respecttively. On average for the two years, on the 14th and 28th, the lowest efficacy was recorded after the treatment with Onix 60 – 1.00 L ha^{-1} – 42.5 and 52.5% respectively. For the other tested herbicide products, the efficacy ranged from 45 to 55% on the 14th day and from 65 to 75% on the 28th day.

Table 2. Efficacy of the herbicides against the weed *S. vernalis*, %

Treatments	2023		2024		Average	
	14 day	28 day	14 day	28 day	14 day	28 day
1. Untreated control	-	-	-	-	-	-
2. Cleranda SC – 2.00 L ha^{-1}	50	70	60	80	55	75
3. Pulsar 40 – 1.20 L ha^{-1}	40	60	50	70	45	65
4. Pulsar Plus – 1.60 L ha^{-1}	45	65	60	75	52.5	70
5. Corum – 1.25 L ha^{-1}	70	90	80	95	75	92.5
6. Onix 60 EC – 1.00 L ha^{-1}	40	50	45	55	42.5	52.5

Table 3 presents the efficacy against *L. serriola*. On average for the period, on the 14th and 28th day after treatments, the highest efficacy was recorded after the application of Corum – 1.25 L ha^{-1} – 75 and 92.5% respectively. On average for the two years of the experiment, the lowest efficacy was recorded after the treatment with Onix 60 EC – 1.00 L ha^{-1} – 37.5 and 47.5% respectively. For the other tested herbicide products, the efficacy ranged from 52.5 to 67.5% on the 14th day and from 77.5 to 87.5% on the 28th day.

Table 3. Efficacy of the herbicides against the weed *L. serriola*, %

Treatments	2023		2024		Average	
	14 day	28 day	14 day	28 day	14 day	28 day
1. Untreated control	-	-	-	-	-	-
2. Cleranda SC – 2.00 L ha^{-1}	60	80	75	95	67.5	87.5
3. Pulsar 40 – 1.20 L ha^{-1}	50	70	55	80	52.5	75
4. Pulsar Plus – 1.60 L ha^{-1}	55	75	65	80	60	77.5
5. Corum – 1.25 L ha^{-1}	70	90	80	95	75	92.5
6. Onix 60 EC – 1.00 L ha^{-1}	35	45	40	50	37.5	47.5

On Table 4 is the efficacy against the weed *C. bursa-pastoris*. The efficacy of the evaluated products against this weed was higher than that found for the abovementioned species.

Table 4. Efficacy of the herbicides against the weed *C. bursa-pastoris*, %

Treatments	2023		2024		Average	
	14 day	28 day	14 day	28 day	14 day	28 day
1. Untreated control	-	-	-	-	-	-
2. Cleranda SC – 2.00 L ha^{-1}	70	90	75	95	72.5	92.5
3. Pulsar 40 – 1.20 L ha^{-1}	65	85	75	90	70	87.5
4. Pulsar Plus – 1.60 L ha^{-1}	75	90	80	85	77.5	87.5
5. Corum – 1.25 L ha^{-1}	75	95	75	95	75	95
6. Onix 60 EC – 1.00 L ha^{-1}	40	50	50	60	45	55

On average for the period, on the 14th and 28th day after treatment, the highest efficacy after the application of Corum – 1.25 L ha^{-1} – 75 and 95% respectively was recorded. The efficacy of the treatment with Pulsar 40 and Pulsar Plus against the weed was high as well. On average for the two years of the trial, on the 14th and 28th day after treatment, the lowest efficacy was recorded after the treatment with Onix 60 EC – 1.00 L ha^{-1} – 45 and 55% respectively.

The efficacy against the weed *Th. arvense* is shown on Table 5. Both for *C. bursa-pastoris* and for *Th. Arvense* as weel, the efficacy of the studied herbicidal products was higher than that recorded for the weeds *S. vernalis* and *L. serriola*. On average for the period, on the 14th and 28th day after treatment, the highest efficacy was recorded after the application of Pulsar Plus – 1.60 L ha^{-1} – 77.5 and 95% respectively. The efficacy of the treatment with Pulsar 40 and Corum against the weed was high – 72.5 and 92.5% respectively on the 14th and 28th day after treatment. On average for the two years of the experiment, the lowest efficacy after the treatment with Onyx 60 EC – 1.00 L ha^{-1} – 37.5 and 47.5% respectively was recorded.

Table 5. Efficacy of the herbicides against the weed *Th. arvense,* %

Treatments	2023		2024		Average	
	14 day	28 day	14 day	28 day	14 day	28 day
1. Untreated control	-	-	-	-	-	-
2. Cleranda SC – 2.00 L ha^{-1}	75	90	70	95	72.5	92.5
3. Pulsar 40 – 1.20 L ha^{-1}	70	85	65	85	67.5	85
4. Pulsar Plus – 1.60 L ha^{-1}	75	95	80	95	77.5	95
5. Corum – 1.25 L ha^{-1}	70	90	75	95	72.5	92.5
6. Onix 60 EC – 1.00 L ha^{-1}	35	45	40	50	37.5	47.5

On Table 6 is presented the efficacy against the weed *S. oleraceus*. On average for the period, the highest efficacy was recorded after the application of Cleranda SK – 2.00 L ha^{-1} – 75 and 97.5% on the 14th and on the 28th day after treatments respectively. The efficacy of the treatment with Pulsar 40, Pulsar Plus and Corum against the weed on the 14th day after treatment was 70%. On the 28th day, the efficacy of Pulsar

40 and Pulsar Plus was 90%, and that of Corum was 97.5%.

Table 6. Efficacy of the herbicides against the weed *S. oleraceus,* %

Treatments	2023		2024		Average	
	14 day	28 day	14 day	28 day	14 day	28 day
1. Untreated control	-	-	-	-	-	-
2. Cleranda SC – 2.00 L ha^{-1}	70	95	80	100	75	97,5
3. Pulsar 40 – 1.20 L ha^{-1}	65	85	75	95	70	90
4. Pulsar Plus – 1.60 L ha^{-1}	75	90	65	90	70	90
5. Corum – 1.25 L ha^{-1}	70	90	70	95	70	92,5
6. Onix 60 EC – 1.00 L ha^{-1}	50	60	50	60	50	60

The efficacy against the weed *D. sophia* is shown on Table 7. On average for the period, on the 14th and 28th day after the herbicidal applications, the highest efficacy after the treatment with Pulsar Plus – 1.60 L ha^{-1} – 87.5 and 100% respectively was recorded. The efficacy of the treatment with Cleranda SC (77.5%), Pulsar 40 (82.5%) and Corum (72.5%) against the weed on the 14th day after treatment was high as well. On the 28th day, the efficacy of Cleranda SC and Pulsar 40 was 97.5%, and that of Corum – 95%. On average for the two years of the experiment, on the 14th and 28th day after treatment, the lowest efficacy was recorded after the treatment with Onyx – 1.00 L ha^{-1} – 50 and 55% respectively.

Table 7. Efficacy of the herbicides against the weed *D. sophia,* %

Treatments	2023		2024		Average	
	14 day	28 day	14 day	28 day	14 day	28 day
1. Untreated control	-	-	-	-	-	-
2. Cleranda SC – 2.00 L ha^{-1}	75	95	80	100	77,5	97,5
3. Pulsar 40 – 1.20 L ha^{-1}	85	100	80	95	82,5	97,5
4. Pulsar Plus – 1.60 L ha^{-1}	85	100	90	100	87,5	100
5. Corum – 1.25 L ha^{-1}	70	95	75	95	72,5	95
6. Onix 60 EC – 1.00 L ha^{-1}	50	55	50	55	50	55

Visual symptoms of phytotoxicity 7 and 14 days after treatment with the herbicides are presented in Table 8. Seven days after the herbicidal praying, the strongest symptoms of phytotoxicity were found at variants 3 (Pulsar 40 – 1.20 L ha^{-1}), 4 (Pulsar Plus – 1.60 L ha^{-1}), and 6 (Onix 60 EC – 1.00 L ha^{-1}) – score 3.

Table 8. Visual phytotoxicity 7 and 14 days after treatments, scores by EWRS

Treatments	2023		2024	
	7 day	14 day	7 day	14 day
1. Untreated control	-	-	-	-
2. Cleranda SC – 2.00 L ha^{-1}	2	1	2	1
3. Pulsar 40 – 1.20 L ha^{-1}	3	1	3	1
4. Pulsar Plus – 1.60 L ha^{-1}	3	1	3	1
5. Corum – 1.25 L ha^{-1}	2	1	2	1
6. Onix 60 EC – 1.00 L ha^{-1}	3	1	3	1

The expressed phytotoxic symptoms of Pulsar 40 and Pulsar Plus were stronger yellowing of the leaves (stronger chlorosis). On the 14th day, the symptoms of phytotoxicity were overcome by the crop. The symptoms caused by the herbicide Onix 60 EC were chlorosis and necrotic spots on the alfalfa leaves. In this variant, the symptoms of phytotoxicity were not completely overcome, and by the second reporting date. On the old leaves of the plants, the symptoms of phytotoxicity remain visible until the time of the first mowing. On the 7th day after the herbicidal spplications, phytotoxicity score of 2 in variants 2 (Cleranda SK – 2.00 L ha^{-1}) and 5 (Corum – 1.25 L ha^{-1}) was found. Slight yellowing of the leaves was observed (weak chlorosis). On the 14th day, the symptoms of phytotoxicity were overcome by the crop.

The obtained data for the chlorophyll content index (CCI) for alfalfa is presented in Table 9. Quantification of pigments in plant leaves using non-invasive optical methods is quick and easy, providing reliable data on the relative chlorophyll content compared to traditional and chemical methods (Richardson et al., 2002). Miri (2009) reported that the chlorophyll content index (CCI) is directly and positively correlated with yields. The chlorophyll content in leaves is also used to measure the nitrogen content in leaves, but also as an important indicator of nitrogen deficiency in plants (Cerovic et al., 2012).

In addition, the chlorophyll content index (CCI) can be used as a tool to support decision-making on nitrogen fertilization of crops, as well as to estimate the yield of cultivated plants. The index varies widely among different crops. Tracking the index over time aims to identify any potential disturbances in plants as a result of the treatments.

Table 9. Chlorophyll content index (CCI)

Treatments	2023			2024		
	7 days	14 days	21 days	7 days	14 days	21 days
1.Untreated control	26.2 a	31.6 c	38.6 b	29.0 a	329 c	36.7 d
2.Cleranda SC – 2.00 L ha^{-1}	24.2 b	35.7 a	42.0 a	22.6 c	36.1 a	44.7 a
3. Pulsar 40 – 1.20 L ha^{-1}	23.2 c	33.5 b	41.9 a	24.4 b	35.9 a	44.9 a
4. Pulsar Plus – 1.60 L ha^{-1}	22.9 c	32.9 b	41.5 a	24.7 b	34.8 b	43.5 b
5. Corum – 1.25 L ha^{-1}	24.0 b	34.1 b	41.6 a	23.9 b	36.2 a	42.6 c
6. Onix 60 EC – 1.00 L ha^{-1}	17.9 d	26.8 d	34.9 c	20.7 d	31.2 d	35.8 e

Figures with different letters are with a proven difference by Duncan's multiple range test ($p<0.05$)

The data obtained from the dynamic measurements showed that the CCI index increases over time. On the first measurement date and in both experimental years, the highest index was recorded in the untreated control 26.2 CCI (2023) and 29.0 CCI (2023). The lowest chlorophyll index was measured in variant 6 (Onyx 60 EC – 1.00 L ha^{-1}) in both experimental years – 17.9 CCI in 2021 and 20.7 CCI in 2022. On the 7th day after treatment, the chlorophyll index in the remaining variants varied from 22.9 to 24.2 CCI.

On the 14th day after treatments, the highest chlorophyll index was established in variant 2 (Cleranda SC – 2.00 L ha^{-1}) in 2023 – 35.7 CCI. In 2024 with proven higher results were variants 2 (Cleranda SC – 2.00 L ha^{-1}), 3 (Pulsar 40 – 1.20 L ha^{-1}) and 5 (Corum – 1.25 L ha^{-1}) – 36.1, 35.9 and 36.2 CCI respectively. The studied parameter in the untreated control was lower on the second measurement date in both years.

On the 21st day after treatment in 2023 with proven higher results for the chlorophyll index compared to the untreated weedy control and option 6 (Onyx – 100 ml/da) are options 2. (Cleranda SC – 2.00 L ha^{-1}), 3 (Pulsar 40 – 1.20 L ha^{-1}), 4 (Pulsar Plus – 1.60 L ha^{-1}) and 5 (Corum – 1.25 L ha^{-1}) - 42.0, 41.9, 41.5 and 41.6 CCI respectively.

On the 21st day after the application of the herbicidal products in 2023 with proven higher results for the chlorophyll index compared to the other variants were variants 2 (Cleranda SC – 2.00 L ha^{-1}) and 3 (Pulsar 40 – 1.20 L ha^{-1}) - 44.7 and 44.9 CCI respectively.

The results obtained for the plant height before the 1st and 2nd swathing of alfalfa are presented in Table 10. Larger differences in plant height were recorded for the first swath. On average for the two experimental years, variant 6 (Onyx 60 EC – 1.00 L ha^{-1}) had the lowest plants – 48.59 cm for the first swath and 51.19 cm for the second swath. The plants from this variant were proven to be lower than the untreated control – 51.92 cm for the first and 51.97 cm for the second swath. In variant 5 (Corum – 1.25 L ha^{-1}) the highest plants were measured – 66.59 cm for the first swath. In the second swath, the plants from variant 3 (Pulsar 40 – 1.20 L ha^{-1}) were the highest – 54.46 cm.

Table 10. Height of the plants at 1st and 2nd swaths, cm

Treatments	2023		2024		Average	
	Swath 1	Swath 2	Swath 1	Swath 2	Swath 1	Swath 2
1. Untreated control	54.71 d	50.12 d	57.12 d	53.22 c	55.92	51.67
2. Cleranda SC – 2.00 L ha^{-1}	62.54 b	52.19 b	65.19 b	54.07 b	63.87	53.33
3. Pulsar 40 – 1.20 L ha^{-1}	61.48 c	53.41 a	64.51 c	55.50 a	63.00	54.46
4. Pulsar Plus – 1.60 L ha^{-1}	60.84 c	51.20 c	63.40 c	52.74 c	6212	51.97
5. Corum – 1.25 L ha^{-1}	65.89 a	52.61 b	67.29 a	53.47 b	66.59	53.04
6. Onix 60 EC – 1.00 L ha^{-1}	49.81 e	50.67 d	47.36 e	51.70 d	48.59	51.19

Figures with different letters are with a proven difference by Duncan's multiple range test ($p<0.05$)

At the plots treated with herbicides, dry matter yields at the first swatrh were 30 to 81% and 11 to 78% of those of the untreated and hand-weeded controls, respectively. At the second swath, alfalfa dry matter yields were not affected by the weed control method (Kunelius, 1974). Idris et al. (2014) found that the reduction in yields due to weed competition is 57% and 37% at the first swath, in the first and second year, respectively. The herbicide treatments significantly increased alfalfa yield compared to the untreated control (Cosgrove and Barrett, 1987, Kostov and Pacanoski 2006; Pacanoski, 2011). Our research data corresponds with the findings of the abovementioned authors.

The obtained data from the current trial is shown on table 11. As in the plant height, in the of fresh matter yield from the first swath, larger differences were recorded as well. On average for the two experimental years, the untreated control had the lowest yield of fresh matter – 11.20 t ha^{-1} in the first cut and 13.30 t ha^{-1} in the second swath.

Table 11. Fresh mass yield from the 1st and 2nd swath, t ha^{-1}

Treatments	2023		Total from the two swaths
	Swath 1	Swath 2	
1. Untreated control	09.80 e	13.60 c	23.40
2. Cleranda SC – 2.00 L ha^{-1}	13.20 c	14.00 a	27.20
3. Pulsar 40 – 1.20 L ha^{-1}	13.50 bc	14.20 a	27.70
4. Pulsar Plus – 1.60 L ha^{-1}	14.00 b	13.80 b	27.80
5. Corum – 1.25 L ha^{-1}	15.10 a	14.50 a	29.60
6. Onix 60 EC – 1.00 L ha^{-1}	11.80 d	14.10 a	25.90
Treatments	2024		Total from the two swaths
	Swath 1	Swath 2	
1. Untreated control	12.70 e	13.00 c	25.70
2. Cleranda SC – 2.00 L ha^{-1}	13.80 d	13.70 ab	27.50
3. Pulsar 40 – 1.20 L ha^{-1}	14.20 c	13.90 ab	28.10
4. Pulsar Plus – 1.60 L ha^{-1}	14.70 b	14.10 a	28.80
5. Corum – 1.25 L ha^{-1}	16.20 a	14.30 a	30.50
6. Onix 60 EC – 1.00 L ha^{-1}	10.50 f	13.80 ab	24.30
Treatments	Average		Total from the two swaths - Average
	Swath 1	Swath 2	
1. Untreated control	11.20	13.30	24.50
2. Cleranda SC – 2.00 L ha^{-1}	13.50	13.90	27.40
3. Pulsar 40 – 1.20 L ha^{-1}	13.90	14.10	27.90
4. Pulsar Plus – 1.60 L ha^{-1}	14.40	14.00	28.30
5. Corum – 1.25 L ha^{-1}	15.70	14.40	30.10
6. Onix 60 EC – 1.00 L ha^{-1}	11.30	14.00	25.20

Figures with different letters are with a proven difference by Duncan's multiple range test ($p<0.05$).

The average total yield of fresh matter in the untreated control was 24.50 t ha^{-1}. Variant 5 (Corum – 1.25 L ha^{-1}) stood out with the highest yields of fresh matter – 16.20 t ha^{-1} in the first and 14.30 t ha^{-1} in the second swath. The average total yield of fresh matter from the two swaths in this variant was 30.10 t ha^{-1}. In the other variants of the experiment, the average total yield of fresh matter from the two cuts varied from 25.20 t ha^{-1} in variant 6 (Onyx 60 EC – 1.00 L ha^{-1}) to 28.30 t ha^{-1} in variant 4 (Pulsar Plus – 1.60 L ha^{-1}).

The results obtained for the dry matter yield at the 1st and 2nd swaths of alfalfa are presented in Table 12. Average for the two trial years with the lowest dry matter yield were the untreated control and variant 5 (Onyx 60 EC – 1.00 L ha^{-1}) – 4.00 t ha^{-1} at the first swath. At the second swath, the lowest dry matter yields were found to be from the untreated control and variant 5. Variant 5 (Corum – 1.25 L ha^{-1}) stood out with the highest dry matter yields – 5.10 t ha^{-1}. The average total dry matter yield from the two swaths in this variant was 9.70 t ha^{-1}.

Table 12. Dry matter yield at the 1st and 2nd swaths of alfalfa

Treatments	2023		Total from the two swaths
	Swath 1	Swath 2	
1. Untreated control	3.80 c	4.10 ab	7.90
2. Cleranda SC – 2.00 L ha^{-1}	4.50 b	4.20 ab	8.70
3. Pulsar 40 – 1.20 L ha^{-1}	4.10 bc	4.50 a	8.60
4. Pulsar Plus – 1.60 L ha^{-1}	4.30 b	4.20 ab	8.50
5. Corum – 1.25 L ha^{-1}	4.90 a	4.30 a	9.20
6. Onix 60 EC – 1.00 L ha^{-1}	3.90 c	4.20 ab	8.10
Treatments	2024		Total from the two swaths
	Swath 1	Swath 2	
1. Untreated control	4.20 c	4.30 c	8.50
2. Cleranda SC – 2.00 L ha^{-1}	4.80 b	4.50 b	9.30
3. Pulsar 40 – 1.20 L ha^{-1}	4.70 b	4.60 b	9.30
4. Pulsar Plus – 1.60 L ha^{-1}	4.80 b	4.60 b	9.40
5. Corum – 1.25 L ha^{-1}	5.20 a	4.90 a	10.10
6. Onix 60 EC – 1.00 L ha^{-1}	4.10 c	4.00 d	8.10
Treatments	Average		Total from the two swaths - Average
	Swath 1	Swath 2	
1. Untreated control	4.00	4.20	8.20
2. Cleranda SC – 2.00 L ha^{-1}	4.70	4.40	9.10
3. Pulsar 40 – 1.20 L ha^{-1}	4.40	4.60	9.00
4. Pulsar Plus – 1.60 L ha^{-1}	4.60	4.40	9.00
5. Corum – 1.25 L ha^{-1}	5.10	4.60	9.70
6. Onix 60 EC – 1.00 L ha^{-1}	4.00	4.10	8.10

Figures with different letters are with a proven difference by Duncan's multiple range test ($p<0.05$).

CONCLUSIONS

The highest efficacy against the weeds *S. vernalis*, *L. serriola* and *C. bursa-pastoris* after the application of Corum was recorded, and the highest efficacy against the weeds *Th. arvense* and *D. sophia* was reported from Pulsar Plus. The highest efficacy against the weed *S. oleraceus* was recorded by Cleranda SC. During

the first measurement date, during both experimental years, the highest chlorophyll index was recorded in the untreated control, and the lowest at Onyx 60 EC. On the following measurement dates in the herbicide-treated variants, with the exception of variant 6, the chlorophyll index increased. In the first swath, variant 6 (Onyx 60 SC) was distinguished by the lowest plants, and variant 5 (Corum) by the highest. In the second swath, the plants of variant 3 (Pulsar 40) were the highest. Variant 5 (Corum) showed the highest yields of fresh and dry alfalfa mass.

ACKNOWLEDGEMENTS

The research was financially supported by Project 17-12 at the Center of Research, Technology Transfer and Protection of Intellectual Property Rights at the Agricultural University of Plovdiv, Bulgaria.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

STUDIES ON THE INFLUENCE OF GROWTH REGULATORS IN REDUCING THE EFFECTS OF CLIMATE CHANGE ON SOYBEAN CULTIVATION IN CENTRAL MOLDAVIA

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Abstract

Climatic stress, such as drought and extreme temperatures, represents a major threat to the growth and development of crops, including soybeans, which require a balanced water regime. Water is an essential component of plant life, and its absence is the most crucial abiotic factor negatively affecting the quality and quantity of agricultural production. According to the specialized literature, growth regulators positively influence the morphology, physiology, and quality of soybean production. Recent studies indicate that applying such products at optimal concentrations and growth stages can mitigate abiotic stress. Based on this premise, a study was conducted in the experimental field at ARDS Secuieni, aiming to determine the influence of growth regulators on the productive potential of soybeans. The experiment was bifactorial type, using two soybean varieties created by ARDS Turda and three growth regulators applied during the flowering period. The results highlighted that the tested regulators influenced the adaptability of the soybean varieties to drought.

Key words*: drought, growth regulators, soybean.*

INTRODUCTION

Soy is one of the most important protein and oilseed crops worldwide. At the same time, the importance of soy is also evident in mixed farming systems, making it a key crop both economically and environmentally. In these systems, soybean contributes not only to the diversification of agricultural production but also to the improvement of soil health, having beneficial effects in a cropland, reducing the need for pesticides and chemical fertilizers (Badea et al., 2023; Joița-Păcureanu et al., 2023). However, Europe relies heavily on imports due to the small area devoted to legume cultivation, representing only 1.5% of agricultural land compared to global needs (Watson et al., 2017).

Phytohormones play a key role in regulating the stress response and enhancing plant growth, with a particular focus on soybean. These hormones interact to trigger specific signaling mechanisms that control gene expression and contribute to plant adaptation to adverse conditions. Abiotic stress, particularly drought, negatively affects crop productivity, and in this context, abscisic acid (ABA) acts as a master regulator in the intense responses, causing stomata to close. In the long term, ABA interacts with other hormones such as jasmonic acid (JA), gibberellins (GA), salicylic acid (SA) and brassinositides (BR) to stimulate stomatal closure by regulating gene expression. In contrast, cytokinins (CK) and auxins (IAA) regulate stomatal opening. Abscisic acid (ABA) is present in various organisms and plays a crucial role in regulating cell growth and stress tolerance. Under water stress, endogenous ABA levels increase rapidly, acting as signaling molecules that regulate genes such as DREB proteins and bZIP transcription factors. ABA induces stomatal closure to prevent water loss and improves root architecture to facilitate access to soil water. Exogenous application of phytohormones can enhance drought tolerance of soybean (Shaffique et al., 2023).

Global demand for the soybean crop is increasing due to its economic and nutritional importance, but production is often affected by abiotic stressors such as drought and salinity, which emphasizes the need for innovative strategies, such as the use of plant growth regulators, to improve crop resilience and ensure sustainable yields. The impact of plant growth regulators (PGRs) on soybean productivity highlights how they influence physiology, morphology and stress tolerance. Through exogenous application, PGRs can improve photosynthetic rate, increase chlorophyll content and stimulate translocation of assimilates, which favors biomass accumulation and yield increase. For example, salicylic acid stabilizes photosystem II, improving water use efficiency and antioxidant activity, while cytokinins and gibberellic acid (GA_3) stimulate cell division and stem elongation, contributing to better plant growth. At the morphological level, PGRs such as indolbutyric acid (IBA) promote root development, improving water and nutrient uptake, and ethephon (ETP) increases root surface area, thus increasing resistance to stress conditions. In addition, these compounds play an essential role in ameliorating the effects of abiotic stresses, such as drought and salinity, by activating the plant's defense mechanisms. Application of methyl jasmonate (MeJA) improves drought tolerance by increasing the concentration of soluble sugars and secondary metabolites involved in stress adaptation. Besides yield enhancement, PGR influences seed quality by contributing to higher protein and oil content (Amoanimaa et al., 2022).

The productivity of soybean crop largely depends on the availability of nitrogen, which significantly influences plant growth and development (Salvagiotti et al., 2008), but with its ability to fix atmospheric nitrogen, soybean becomes an important crop in crop rotations, helping to maintain soil fertility and reduce the need for chemical fertilizers. Thus, soybean not only improves agricultural crop yields, but also brings environmental benefits by improving soil structure and nutrient cycling (Ionescu et al., 2022; Moldovan et al., 2022).

Although the application of plant growth regulators (PGRs) has been limited in the past, analysis of the role of the synthetic cytokinin cytokini 6-benzyladenine (6-BAP) in improving drought tolerance of soybean highlights the benefits of exogenous use on plant growth and productivity. Drought adversely affects soybean growth, causing cell dehydration, reduced water potential, accumulation of reactive oxygen species and accelerated senescence, leading to decreased productivity. The 6-BAP molecule contributes to regulating cell division, delaying senescence, improving root architecture and stimulating the translocation of photosynthesis products, thus favoring better adaptation to water stress conditions. In addition, exogenous application of 6-BAP at the end of flowering reduces floral abortion and increases the number of pods and seeds, significantly improving yield. It also plays a key role in plant adaptation to fluctuating water availability, helping to maintain crop vitality (Mangena Phetole, 2022).

Analysis of the effects of the application of a plant growth regulator (PGR) on soybeans at the R2 (beginning of pod formation) and R3 (pod formation) stages, highlights its impact on yield and agronomic characteristics of the crop. Growth regulators are chemicals that influence plant physiological processes such as cell division, stem elongation and stress tolerance. Among the most important PGRs involved in soybean growth are gibberellic acid (GA_3), cytokinins and salicylic acid. Gibberellic acid stimulates stem elongation, fruit development and pod formation, leading to more seeds per plant. Cytokinins, such as kinetin, regulate cell division and delay senescence, having a positive effect on seed weight and quality, in particular by increasing protein content. Salicylic acid plays a key role in stress adaptation, influencing stomatal closure and improving the harvest index, which contributes to increased yield. The study demonstrates that the application of growth regulators in R2-R3 phases can significantly improve the yield and quality of soybean crops, but the efficacy of these products depends on the type of PGR used and the optimal timing of application (Klumpp Geoffrey, 2018).

The impact of plant growth regulators (PGRs) on nitrogen metabolism, yield and quality of the symbiosis between soybean and nitrogen-fixing bacteria was analyzed using various PGRs types (auxins, cytokinins, gibberellins and mepiquat chloride). These were applied to soybean plants following batting to assess the effects on

nitrogen fixation and plant growth. The results showed that the application of these PGRs significantly stimulated the activity of symbiotic batteries, improving the atmospheric nitrogen fixation process, and consequently the growth and development of soybean crop. Treated plants showed higher yield with better nodule development and increased nitrogen concentration. In addition, PGRs positively influenced nitrogen metabolism by stimulating enzyme activity associated with nitrogen assimilation and utilization in plant metabolic processes. Seed quality was also improved, with an increase in protein and lipid content, suggesting a higher efficiency in the utilization of nitrogen resources and a positive impact on the nutritional value of the seeds. Accordingly, the use of growth regulators can optimize the symbiosis between soybean and nitrogen-fixing bacteria, thereby improving the quantity and quality of soybean crops under more sustainable conditions and with less consumption of chemical fertilizers (Chen et al., 2023).

Based on these hypotheses, this paper highlights how commercial products applied on soybean plants have influenced productivity elements in the context of climate change in Central Moldova.

MATERIALS AND METHODS

This paper presents the partial results of an experiment that was located in the experimental field of A.R.D.S. Secuieni, in the year 2023. It aimed to analyze the influence of some growth regulators on some productivity elements of soybean plants. The experiment was bifactorial, where factor A was represented by the soybean variety and factor B by the commercial product. Factor A had 2 gradations, represented by the varieties Onix and Ziana TD, and factor B had 4 gradations, the untreated control and the products Moddus Evo, Ormet and Toprex.

Both Onix and Ziana TD are Romanian varieties created and patented by A.R.D.S. Turda. Varieties are early maturing, falling in the OO maturity group, with Ziana TD being on the borderline between OOO and OO. These varieties have a high yield potential, reaching 4600 kg/ha for Onix and 5000 kg/ha for Ziana TD. Their grains have a protein content of over 40% and a lipid content between 20.9% and 24.6%, ensuring superior nutritional quality. Also, both varieties show very good resistance to lodging, lodging and lodging, and are recommended for the favorable soybean areas of Transylvania, Moldova and the Western Plain.

To fulfill the research objectives set, both soybean varieties were treated with 3 different commercial products with specific growth regulator action.

The physiological action of these products consists in modifying the morpho-physiological characteristics of the plants. They inhibit stem length growth, in particular by reducing internode elongation. At the same time, they accelerate the flowering, fruiting, and fruit ripening processes. At the same time, they contribute to increasing plant tolerance to stress factors such as drought, salinity, diseases, and pests by limiting cell extension and stimulating tissue differentiation (Jităreanu & Toma, 2007).

Moddus Evo is a growth regulator containing 250 g/l trinexapac-ethyl and is applied to control plant elongation, improving plant stability and reducing the risk of plant drop. It is rapidly absorbed through leaves and stems, acting on meristems to inhibit internode elongation. The use of this product results in a more robust plant with a reduced risk of damage in high winds or heavy rain (https://www.syngenta.ro/product/crop-protection/moddus-evo).

Ormet is a growth regulator containing 480 g/l ethephon that regulates the elongation of plants, helping them to become more stable and more resistant to environmental factors such as wind and heavy rain. It is rapidly absorbed and translocated to the meristematic zones, where it inhibits excessive stem elongation and helps to strengthen the straw. It is used in fall and spring wheat and barley crops and is applied from the second internode to the emergence of the spikelets, depending on the phenophyse of the plant. Ormet thus helps to reduce the risk of plant drop and promotes a quality harvest (https://www.adama.com/romania/ro/produse-pentru-protectia-plantelor/regulatori-crestere/ormet).

Toprex is a combined fungicide and growth regulator containing 20 g/l difenoconazole and 125 g/l paclobutrazol, effective in both disease control and improving plant stability. It helps reduce the risk of oilseed rape plants falling by

stimulating a healthy root system and inhibiting excessive stem elongation. It is applied to oilseed rape crops both in the fall (four-leaf to winter) and in the spring (during stem elongation to the green flower bud). In addition, Toprex protects the crop from *Phoma lingam*, a pathogen that causes black rot of oilseed rape (https://www.adama.com/romania/ro/produse-pentru-protectia-plantelor/fungicide/toprex).

The experiment was set up according to the method of plots subdivided into three repetitions, on a faeozoic soil type (typical Cambian chernozemic) characterized by moderate fertility and physico-chemical properties favorable to agricultural crops. The soil has a neutral pH of 7.29 and a loamy texture. The humus level is 2.3%, indicating good fertility. In terms of nutrients, the soil is poorly supplied with total nitrogen (9.4 mg/kg), but very well supplied with mobile phosphorus (189 mg/kg) and mobile potassium (304 mg/kg), thus ensuring optimal conditions for the development of soybean crops.

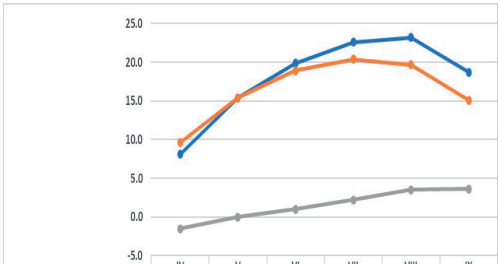
The experiment was set up following all the technological links specific to the soybean crop, the difference being the two treatments applied in the flowering phenophase. The first treatment was applied at the beginning of flowering and the second at full flowering.

In order to highlight the influence of the applied products on the reduction of water stress, determinations were carried out on the number of pods per plant, the number and weight of grains per plant, the thousand kernel weight (TKW), and the yield.

RESULTS AND DISCUSSIONS

The year 2023 was characterized by atypical temperatures for the soybean crop, significantly influencing its development. In April and May, temperatures were at or below the multi-year average (-1.5°C in April and 0°C in May), resulting in slower germination and more difficult initial development. Although soybeans managed to continue their vegetative cycle, this early delay influenced pod formation. Instead, in June, temperatures approached optimal values, favoring vegetative growth and flowering and providing good conditions for further crop development.

However, from July onwards, temperatures deviated significantly above the multi-year average (+2.2°C in July and +3.5°C in August), which caused high heat stress on the plants (Figure 1). Temperatures above 24°C during this period led to flower abortion and poor pod formation, thus limiting yield potential.



	IV	V	VI	VII	VIII	IX
The monthly average (°C)	8.1	15.4	19.9	22.6	23.2	18.7
The multi-year average (°C)	9.6	15.4	18.9	20.4	19.7	15.1
The deviation	-1.5	0.0	1.0	2.2	3.5	3.6

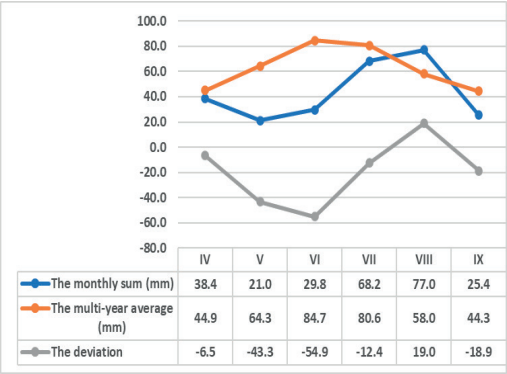
Figure 1. Temperature registrated at A.R.D.S. Secuieni during soybean vegetation period, 2023

In terms of rainfall, 2023 was characterized by a severe rainfall deficit, significantly affecting soybean development. In April, May, and June, rainfall amounts were well below the multi-year average (-6.5 mm, -43.3 mm and -54.9 mm), which created a pronounced water stress in the early growth phases. This shortage hindered the proper development of the root system, limiting the ability of the plants to take up the necessary water during critical periods. In July, the deficit continued (-12.4mm), amplifying the impact of high temperatures and directly affecting pod formation and berry filling. The only month with rainfall above multi-year values was August.

In September, rainfall again fell below the multi-year average (-18.9 mm), accelerating ripening but also contributing to uneven ripening (Figure 2).

Thus, the lack of rainfall, coupled with high temperatures, resulted in a very dry growing season, limiting soybean yield and quality.

The number of pods/plant is a key determinant and is directly related to the ability of the plant to produce seed. This parameter is influenced by environmental conditions, nutrition and applied treatments such as growth regulators. Figure 3 shows that the treatments applied positively influenced pod formation on the plant.



	IV	V	VI	VII	VIII	IX
The monthly sum (mm)	38.4	21.0	29.8	68.2	77.0	25.4
The multi-yeaar average (mm)	44.9	64.3	84.7	80.6	58.0	44.3
The deviation	-6.5	-43.3	-54.9	-12.4	19.0	-18.9

Figure 2. Rainfall registrated at A.R.D.S. Secuieni during soybean vegetation period, 2023

In the case of Onix, the highest average number of pods/plant was recorded in the version with Ormet, followed by Moddus Evo. The control had a similar value with the third growth regulator, about 68 pods/plant.

The Ziana TD variety had lower pod number averages compared to Onix. However, in plots where treatments were performed, higher values were determined than the control sample. This time, the highest average number of pods/plant was influenced by the Toprex product (58.6), followed by Ormet (45.13) and then Moddus Evo (Figure 3).

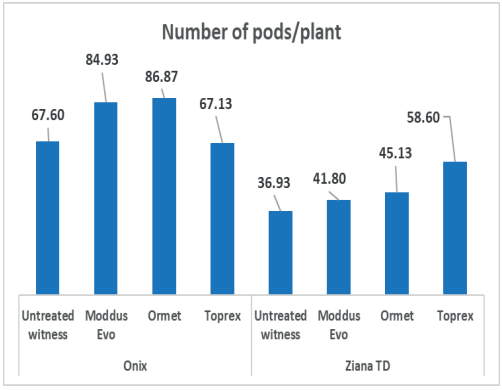


Figure 3. Average number of pods per plant

The number of grains per plant is another important factor in assessing the yield potential of soybeans. It is influenced by the plant's ability to fertilize and develop seeds, a process that can be stimulated or inhibited by growth regulators. More kernels per plant indicate a higher yield, while fewer kernels per plant may signal problems with fertilization and stress conditions.

Directly related to the factor analyzed above, the number of kernels per plant follows a similar trend.

In Onix, average values ranged from 130.73 grains/plant (Toprex) to 181.7 grains/plant (Moddus Evo).

Ziana TD also showed significant differences between treated and control variants. The plot treated with the growth regulator Toprex (114.53) showed the highest average number of grains/plant, while the control plot (76.4) showed the lowest average number of grains/plant (Figure 4).

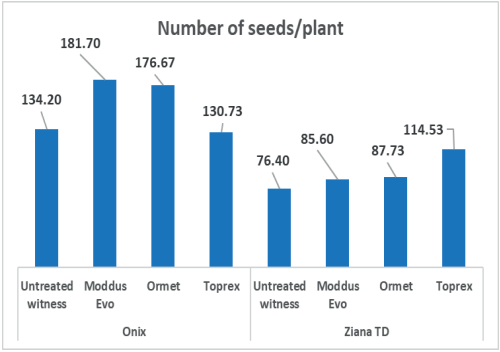


Figure 4. Average number of seeds per plant

The weight of the seeds per plant is an important factor in assessing yield potential, with heavier berries indicating adequate nutrition and optimal plant development.

In the case of Onix, the highest weight of the seeds per plant was 19.52 g (Moddus Evo), followed by the variety with Ormet (17.26 g). The plot sprayed with the Toprex regulator had a slightly lower average weight than the control (14.24 g) and 13.75 g, respectively.

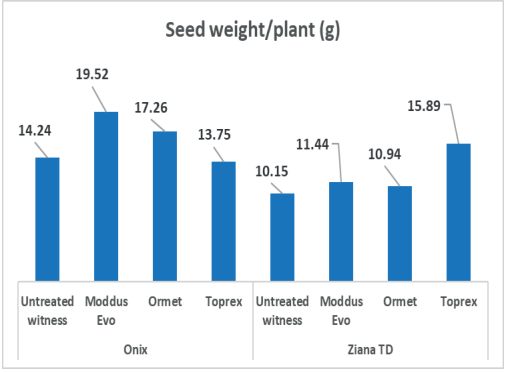


Figure 5. Average seed weight per plant (g)

Ziana TD showed values higher than the control in all the variants treated with the three commercial products. Of these, the one in which the Toprex regulator was applied stands out, with an average grain weight of 15.89 g.

While the highest values for the indices analyzed above were determined for the Onix variety, the thousand kernel weight peaked at 143.73 g for Ziana TD, sprayed with Toprex. In the remaining variants, the thousand kernel weight was lower than the control for both varieties. They ranged from 106.99 g (variety Onix treated with Ormet) to 141.9 g (variety Ziana TD, control var. Ziana TD) (Figure 6).

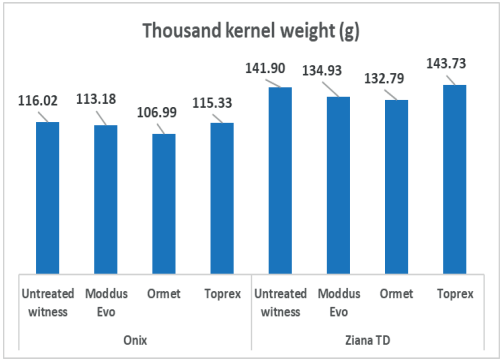


Figure 6. Thousand kernel weight (g) at the soybean, depending on the applied product

In the context of climate change, soybean yield is increasingly affected by extreme weather conditions, such as rainfall deficits and temperature fluctuations. These conditions can significantly reduce the quantity and quality of the harvest, with a direct impact on the availability of soybeans for consumption and industry.

The application of growth regulators can play an important role in plant adaptation to these unfavorable conditions by stimulating physiological processes that help reduce the effects of water stress.

Analyzing Table 1, it can be seen that yields varied both by variety and by product applied.

The highest yield was 1704.33 kg • ha^{-1} for the Onix variety treated with Moddus Evo. This had the highest yield increase of 177.67 kg • ha^{-1} compared to the control sample. At the opposite pole, the Ormet treated variety yielded 1424 kg • ha^{-1}, 102.67 kg • ha^{-1} less than the untreated plot.

In the case of Ziana TD, yields were higher than in the Onix variety. This time, the Toprex-treated variety was more productive than the control, with 1937.33 kg • ha^{-1}. In comparison with the untreated control, the yield increase was 135.67 kg • ha^{-1} (Toprex) and 123 kg • ha^{-1} (Ormet). While the influence of the growth regulator Moddus Evo resulted in a yield increase in Onix, Ziana TD yield was 1746.67 kg • ha^{-1}, 55 kg • ha^{-1} less than the untreated sample. The yield was statistically interpreted, but the differences recorded are not statistically assured (Table 1).

Table 1. Soybean grain production (kg • ha^{-1})

Variety	Treatment	Production (kg/ha)	% compared to control	Difference (kg/ha)	Significance
Onix	Untreated witness	1526.67	100	-	
	Moddus Evo	1704.33	112	177.67	
	Ormet	1424.00	93	-102.67	
	Toprex	1541.67	101	15.00	
Ziana TD	Untreated witness	1801.67	100	-	
	Moddus Evo	1746.67	97	-55.00	
	Ormet	1924.67	107	123.00	
	Toprex	1937.33	108	135.67	
DL kg/ha			5% =	335.8	
			1% =	471.3	
			0.1% =	665.3	

CONCLUSIONS

In climatic terms, the soybean growing season was marked by a precipitation deficit of -117.00 mm, against a 1.5°C increase in temperature compared to multi-year averages. The soybean growing season is characterized as hot and very dry.

Growth regulators had a positive impact on the analyzed productivity indices. They influenced the morpho-physiological processes of the two varieties differently.

The variants in which the commercial product Toprex was applied recorded the highest average values of pod number/plant, number of grains/plant and grain weight/plant for Ziana TD and the lowest for Onix.

Additionally, the highest thousand kernel weight was recorded for the Ziana TD variety treated with Toprex, reaching 143.73 g.

The yield ranged from 1424 kg • ha^{-1}(Onix treated with Ormet) to 1937.33 kg • ha^{-1} (Ziana TD treated with Toprex). Differences in yield compared to the control ranged from -102.67 kg • ha^{-1} (Onix treated with Ormet) to 177.67 kg • ha^{-1} (Onix treated with Moddus Evo).

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

UNVEILING THE EFFECTS OF DROUGHT STRESS ON MAIZE HYBRIDS: CHANGES IN CHLOROPHYLL CONTENT, MORPHOLOGICAL TRAITS AND SOIL-PLANT WATER DYNAMICS

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Abstract

This experiment aimed to assess chlorophyll content under drought conditions in two maize hybrids, KWS Inteligens and Kashmir, within microcosms. The parameters analyzed included chlorophyll content, soil moisture, morphological and growth characteristics based on the BBCH scale (Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie), leaf area, final dry biomass and soil-plant water budget. Chlorophyll content and soil moisture were monitored twice weekly for normal hydric regime (RHN) and for drought or hydric stress (SH). Among the findings, Kashmir hybrid recorded the highest average chlorophyll content of 170 SPAD units after 20 days under RHN, while Inteligens hybrid showed the greatest increase in leaf area of about 2.24 dm². In case of dry biomass accumulated by Kashmir hybrid, a reduction of 63% was achieved under drought compared with RHN, while for Inger this percent was higher with 3%. The results highlight that both hybrids endured drought conditions for up to 23 days from emergence to wilting.

Key words*: BBCH scale, soil-plant water budget, KWS Inteligens, KWS Kashmir, water stress.*

INTRODUCTION

In Romania, maize is the most extensively cultivated crop (Popescu, 2018). As one of the most important cereals for both human and animal consumption, it is primarily grown for grain and animal feed (Dinca et al., 2020). Within the European Community, Romania holds 30% of the total area cultivated with maize. According to FAOSTAT statistics for 2019, Romania had 2,691,930 hectares of maize cultivation, with an average yield of approximately 6.5 t ha^{-1} (64,999 hg ha^{-1}), and a total production of 17,432,220 tons per year (FAOSTAT, 2019). Each year, at the regional scale the frequency and intensity of droughts are increasing (Mustafa et al., 2023). In general plants fresh biomass comprise between 85-90% water for a functional metabolism and assuring integrity (Luqman et al., 2023). These data highlight the significance of maize cultivation and demonstrate its suitability for the eco-pedo-climatic conditions in Romania. Over time, maize has become one of the most widespread and adaptable agricultural crops globally (Ranum et al., 2014). Today, maize is cultivated across the entire globe, from tropical plains to temperate regions and even at high altitudes. The crop's characteristics are closely tied to temperature and precipitation during the growing season (Vâtcă et al., 2021). Climate change has emerged as the greatest global challenge, posing threats particularly to crop patterns and yields (Vâtcă et al., 2021), thereby endangering food security for a growing population (Bennetzen et al., 2016; Godfray et al., 2010). According to the fifth assessment report of the Intergovernmental Panel on Climate Change, climatic conditions are expected to alter the spatial distribution of crops worldwide (Kogo et al., 2019).

Establishing climate risks for individual crops on a global scale and their future evolution is a key requirement for developing a resilient and robust food production system that ensures food security (FAO, 2001; Shirley et al., 2020). Beyond its economic importance, maize plays a crucial role in human nutrition and global food security (Tanumihardjo et al., 2020). As a rich source of carbohydrates, fiber, vitamins, and

minerals, maize is consumed in a wide variety of forms, from fresh kernels to processed products (Ranum et al., 2014).

In many countries, maize is a staple food for millions of people, underscoring its importance in providing sustenance for vulnerable communities (World Health Organization, 2020). One of the greatest challenges facing contemporary agriculture is water stress (Rosegrant et al., 2009), a phenomenon exacerbated by climate change and intensive exploitation of natural resources (Maja and Ayano, 2021). In this context, maize, as a fundamental agricultural crop, is subjected to significant pressures, being at risk of yield deterioration and productivity decline under water scarcity conditions.

Assessing chlorophyll content in maize under such conditions is thus essential for understanding plant adaptation mechanisms to water stress and developing agricultural management strategies that ensure crop sustainability and resilience.

Chlorophyll is the pigment responsible for photosynthesis, the vital process through which plants convert sunlight into chemical energy (Mandal and Dutta, 2020). Under water stress conditions, chlorophyll content may be affected, and evaluating this aspect can provide valuable insights into plant health and adaptability to water-deficient environments (Rosero et al., 2020; Pour-Aboughadareh et al., 2019; Qiao et al., 2024). Some results are contradictory regarding the effect of water stress on chlorophyll content. Findings declared decreased values of chlorophyll contend under drought (Khayatnezhad & Gholamin, 2012; Pour-Aboughadareh et al., 2019; Qiao et al., 2024) or increased values (Rosero et al., 2020). When the values of chlorophyll content are increased under drought stress conditions results that the maize hybrids are resistant to drought conditions (Khayatnezhad & Gholamin, 2012).

The general aim of the study was established to highlight the effects of drought on two KWS maize hybrids. Adhesive objectives were represented by the assessment of (i) experimental factors hybrid, water regime, assessment date effect on chlorophyll content dynamic; (ii) the influence of soil water pool upon chlorophyll content values of both maize hybrids (iii) highlighting the soil water budget in different time-assessments within the experimentation; (iv) correlation coefficient among all parameters from the experiment and (v) providing recommendations regarding maize hybrid best performance under drought.

MATERIALS AND METHODS

Experimental set-up: The experimental protocol consists of 4 treatments in three replicates, therefore each hybrid tested was subjected to normal water regime and also to water stress (drought) in controlled conditions. Thus, T1-T3 included the replications for KWS Inteligens maize hybrid in a normal water regime, T4-T6 the KWS Inteligens hybrid in water stress, T7-T9 the KWS Kashmir hybrid in a normal water regime and T10-T12 the KWS Kashmir hybrid in water stress (Figure 1). The microcosms selected for the experiment had a diameter of 15 cm, and a height of 11 cm.

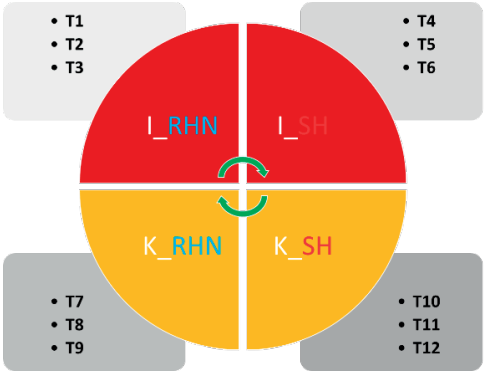


Figure 1. Experimental design. Note: T1-12 - experimental treatments (with replications), RHN - normal hydric regime, SH-hidric stress, drought, I - maize hybrid Inteligens, K - maize hybrid Kashmir

Growth substrate: In each microcosm 500 g soil was introduced. The growth substrate used was a universal soil with a chemical composition as follows: nitrogen (N) 225 mg/l, phosphate (P_2O_5) 125 mg/l, potassium oxide (K_2O) 125 mg/l, with a pH of 7.5, KCl salts below 3 g/l and 57% organic matter, with moisture content of 60%. The RHN was maintained by adding weekly an amount of 200 ml tap water.

Features of maize seeds and tested hybrids: Maize seeds tested were aquired from the Turda Agricultural Research and Development

Station (ARDS Turda - https://scdaturda.ro/). The KWS Inteligens maize hybrid, classified as a PLUS4GRAIN hybrid from the FAO 420 group (mid-late) it is recommended for early sowing. The high production potential is supported by an erect foliage and an excellent ability to stay green, which contributes to efficient photosynthesis and the accumulation of nutrients in the grains. The KWS Kashmir maize hybrid is part of the FAO 370 group, being a mid-early, simple hybrid. Adaptability to various climate and soil conditions is a defining feature of the KWS Kashmir hybrid. One of the main advantages of the KWS Kashmir hybrid is its high production potential. Adaptability to various climate and soil conditions represents also a defining feature of the KWS Kashmir hybrid. The seeds of maize KWS hybrid Kashmir had at the begging of the experiment the average large diameter of 1.15±0.06 cm (SE) and small diameter of 0.67±0.06 cm. The seeds of maize KWS hybrid Inteligens had at the begging of the experiment the average large diameter of 1.15±0.12 cm and small diameter of 1.00±0.61 cm. A number of 10 seeds were placed in each microcosm. Field density recommendations for both maize hybrids are depent on environmental conditions: 65,000 plants/ha in arid areas and 70,000 plants/ha in favourable areas.

Assessments during the experimental trial: Plant physiological monitoring was performed every 2 weeks, from germination to wilting, consisting of measuring the chlorophyll content from the leaves using the MC-100-Apogee Instruments chlorophyll meter (Zlatev et al., 2023). The percentage of soil moisture was done at the time of chlorophyll content readings using the SM-150-Soil moisture kit hygrometer.

Leaf area was measured according the Caliper method and calculated following Anikeev-Kutuzov formula (Nasiyev et al., 2022). Plant growth parameters were identified according to the BBCH phenological scale (Meier, 2018). Plant growth evolution was performed twice a week, administering 200 ml of water only to hybrids under normal water regime, to test the growth and development capacity of the other plants subjected to water stress under drought conditions. At the end of the experiment, the green aboveground biomass was sampled from each microcosm in aluminium containers to determine the plants dry biomass after drying in an oven at 105°C for 24 h until constant weight (Portillo-Estrada et al., 2015). Data analysis: Statistics on the entire dataset were obtained with the open-source RStudio program (R Core Team, 2020), with the package "psych for obtaining and extracting the basic statistics values (Corcoz et al., 2022; Revelle, 2017; Stoian et al., 2022). The package "agricolae" (de Mendiburu, 2019) and "broom" (Robinson, 2014) was used to extract and export three-way (*hypothesis (i)*) and two-way (*hypothesis (ii-iii)*) ANOVA and Least Significant Differences (LSD) test (Stoian et al., 2016; Sângeorzan et al., 2024). For the extraction of correlogram (*hypothesis (iv)*) between all obtained parameters, the package "corrplot" (Wei, 2017) was used. To analyze the effect of treatments on chlorophyll, following *hypothesis (ii)*, boxplots were created using the online platform - https://www.statskingdom.com/, by selecting the advanced box plot graph where are visible all the means, medians, quartile 1, quartile 3, the minimum and maximum values obtained from all repetition assessed.

The pye chart figures were selected to represent all five BBCH scale assessment based on evolution under tested water regimes. The big and first pye chart were chosen to represent the total average percent of germinated seeds from each of the four treatments. Different BBCH sub-stades are projected in the right wit and present the corressponding average percentages for all treatments in evotution. Following all statistical values, figures and correlations the *hypothesis (v)* is accomplished as a conclusion of the study. The formulas used in the experiment:

Maize plants moisture content at the end of experiment – MWC = (FB-DB)/DB*100, where: MWC = maximum plant moisture content at the end on the experiment (Sala et al., 2007), FB = fresh biomass, DB = dry biomass;

Soil moisture content at the end of the experiment – SWC = (HS-DS)/DS*100,

where: SWC = soil moisture content, HS = humid soil, DS = dry soil. The mass of dry soil material was considered as a reference state (Yolcubal et al., 2004).

RESULTS AND DISCUSSIONS

Significant differences ($p<0.001$) were seen between the individual experimental factors - water regime treatments - and different assessments dates (Table 1). Also, the interaction between water regime and different assessment dated had significantly influence the results presenting leaf chlorophyll content and soil moisture content. The triple interaction of all three factors analysed together did not show a significant effect on the obtained results.

Table 1. Three way ANOVA of experimental factors and their impact on the differences between chlorophyll content (C) and soil moisture content (SH)

	C		SH	
	F	*p val*	F	*p val*
H	2.68	0.103	0.57	0.453
W	47.47	<0.001	297.21	<0.001
A	155.99	<0.001	18.31	<0.001
H:W	0.55	0.458	1.23	0.269
H:A	0.18	0.668	0.07	0.795
W:A	27.07	<0.001	12.24	0.001
H:W:A	0.29	0.591	0.33	0.566

Legend: H - maize hybrid; W - water regime;A - assessment date.

The chlorophyll content of maize hybrid leaves is direct dependent on soil moisture content (Figure 2). The highest average value of 202 SPAD units was observed at the maize hybrids from drought (SH) in the second assessment conditions. This value was significantly higher compared to RHN from the same date and also when compared with almost all treatments and assessment dates except first assessment from RHN and third assessment of SH and RHN. The chlorophyll content average values were in the range of 144-178 SPAD units in case of assessment 2-5 from RHN treatments.

Soil moisture content was highest at the beginning of the experiment in RHN treatment, especially at first assessment (Figure 3). Because of drought conditions simulated in SH treatments, the most lower values were observed at the assessments 5 and 6. These values were significantly lower compared to the percentages of soil moisture content from the treatments SH on the assessment dates range between 2-4. Given the fact that at the beginning of the experiment the soil moisture content started from 60%, the SH_1 from first assessment was grouped with the treatments RHN 2, 3 and 5. Germination in the field is very well represented by the results from this experiment in controlled conditions.

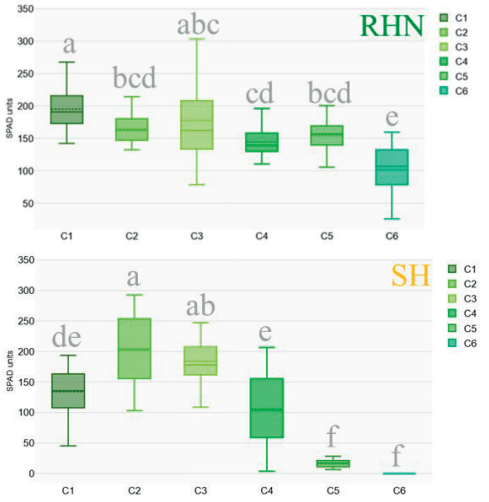


Figure 2. Differences induced by water regime on chlorophyll content values recorded in six assessment dates C1-C6. RHN-normal hydric regime and SH-hydric stress-drought. Different letters between assessment days represent LSD test significant differences at p<0.05

The maize plant growth was differently depending on different individual maize hybrid treatment (Figure 4). At wilting point of the maize plant in the sixth assessment, the plants were in the same BBCH as in the fifth one. The percentage of the total germinated seeds from the treatments with both maize hybrid and water regime increased progressively during the experimental trial.

At the first assessment all the maize seeds reached BBCH 09 stage highlighting the emergence time, the cracking stage, the moment when coleoptile penetrates the soil surface and also the germination percentage. At the second assessment the maize seedlings had the highest share of 47% from the total germinated seeds were in the most advanced BBCH 12 stage for Inteligens hybrid under RHN. This hybrid had the most higher percentages until the end of the experiment regarding seedling development. The maize seedling had 3 unfolded leaves (BBCH 13 stage) at the third and fourth assessment dates.

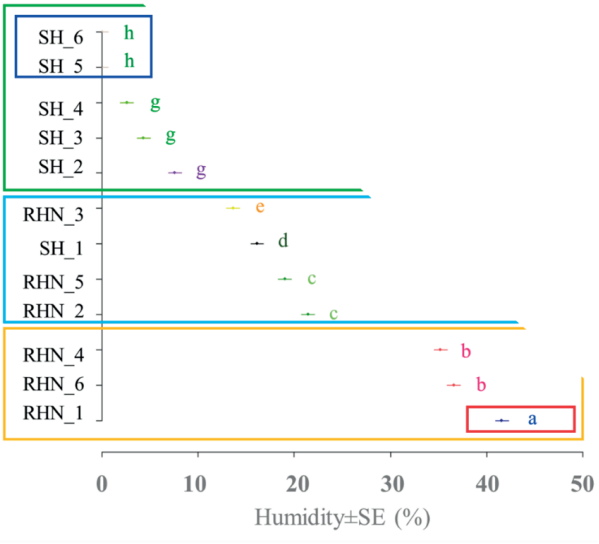


Figure 3. Soil moisture content from RHN-normal hydric regime and SH-hydric stress-drought, at all six assessments independent on the maize hybrid

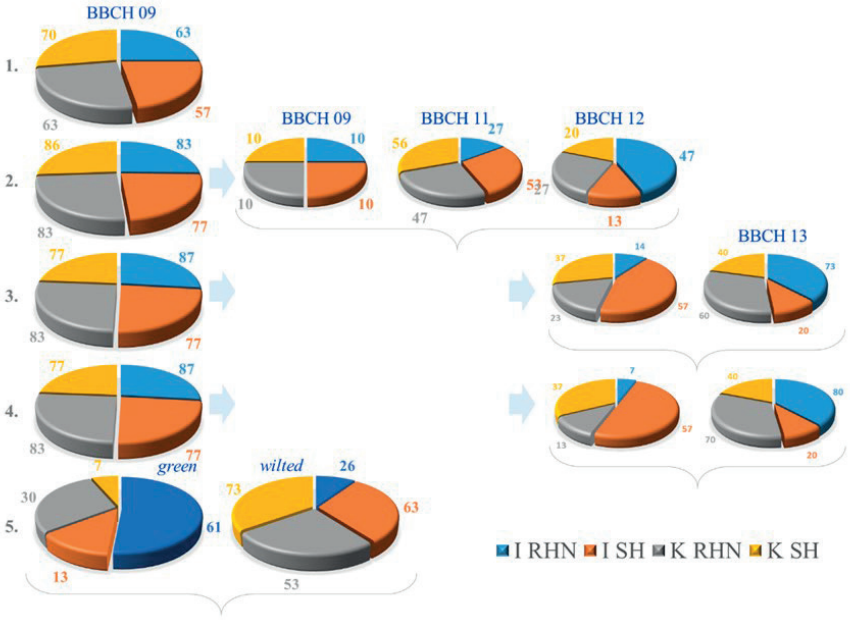


Figure 4. Maize hybrids development under RHN-normal hydric regime and SH-hydric stress-drought treatments on BBCH scale. First chart from all 5 assessments represents total germinated percentages independently on the secondary stages and the following charts represent seeds and seedlings in representative BBCH stage as percentages from the total germinated seeds-first chart. Inteligens maize hybrid - I, Kashmir maize hybrid - K

At the fourth assessment, the Inteligens maize hybrid from RHN treatment increased the percentage of seedlings in BBCH 13 stage with 17% compared with the third assessment. At the last assessment the green and wilted maize seedlings highlighted Inger in RHN more resilient compared to Kashmir hybrid in RHN and Kashmir less resilient to drought conditions

with 10% more wilted plants compared to Inteligens hybrid in SH treatment respectively under drought conditions.

The two-way ANOVA revealed differences between leaf chlorophyll induced by the interaction of water regime and the assessments 1 ($p<0.01$), 5 and 6 ($p<0.001$) (Table 2). Neither the hybrid factor and the interaction between the hybrid and water regime did not determine significant differences between assessments regarding this parameter. The general trend is represented by a reduction of this parameter under drought conditions for both tested hybrids (Alvi et al., 2022; Jan et al., 2024). After 20 days of experimentation, drastic reduction was seen for Inteligens with 138 less SPAD value. For Kashmir were observed with 151 less SPAD units in drought conditions, with the statement that this hybrid recorded higher values for the treatments RHN with around 12% and SH with around 28% compared to Inteligens. Kashmir maize hybrid at the assessment 5 close to the wilting point the highest chlorophyll content of 170 SPAD units.

Table 2. Chlorophyll content difference between tested treatments to all six assessments

		C1	C2	C3	C4	C5	C6
I RHN		216.18±19.27 a	151.5±4.48 a	212.59±29.91 a	147.69±1.04 a	152.32±5.39 a	121.96±9.44 a
I SH		138.81±14.43 b	212.4±46.65 a	201.33±17.08 a	103.33±42.98 a	14.11±5.05 b	0±0 b
K RHN		186.34±3.84 ab	176.87±4.81 a	143.29±12.86 a	141.13±9.08 a	169.91±20.21 a	81±22.42 a
K SH		129.24±17.51 b	191.69±20.96 a	184.16±17.8 a	107.84±22.61 a	18.13±1.39 b	0±0 b
H	F	1.72	0.01	4.48	0.00	1.00	2.83
	p val	*0.226*	*0.930*	*0.067*	*0.968*	*0.345*	*0.131*
W	F	20.07	2.16	0.53	2.47	180.95	69.61
	p val	*0.002*	*0.180*	*0.489*	*0.155*	*<0.001*	*<0.001*
H:W	F	0.46	0.80	1.63	0.05	0.40	2.83
	p val	0.519	0.398	0.238	0.828	0.547	0.131

Note: Different letters indicate significant differences between treatments at each individual of all six assessments.
Legend: H - maize hybrid, W - water regime, RHN - normal hydric regime, SH - hydric stress, C - chlorophyll content 1-6- all six assessments, I - Inteligens maize hybrid, K - Kashmir maize hybrid.

Soil humidity highlights the significant changes depending on the interaction between maize hybrid and water regime (Table 3). The maize seedlings consumed from first assessment to the second 20% from soil humidity due to the leaf developmental stage. The BBCH 12 on which the plants were is part of the most important phenophases for maize when the water requirement is increased (***FAO). In the field conditions it is considered that this growth phase only use the water stored in the soil during winter time and maize crop does not need water from precipitation to emerge from soil and develop the first leaves (Jorda et al., 2022; Șimon et al., 2023). From the second to the third assessment, Inteligens hybrid needed 9% from the soil humidity and Kashmir 7% in RHN. This result highlights that Kashmir could use more efficiently the soil humidity however with a slow development compared to Inteligens. After wetting, at the fourth assessment the soil humidity had high values on Inteligens in RHN treatment had 80% from the total plants in BBCH 13 with three unfolded leaves at the fourth assessment, proving efficient soil water use (soil humidity 37%) (Figure 4). Kashmir in RHN had 70% from total plants in BBCH 13 with low soil humidity around 34%. This trend is suitable also for the fifth assessment of soil humidity. Here Inteligens hybrid from drought conditions still registered very low soil humidity percentage of 0.07% compared to Kashmir where the soil was completely dry.

Table 3. Soil humidity difference between tested treatments to all six assessments

		SH1	SH2	SH3	SH4	SH5	SH6
I_RHN		43.42±1.93 a	23.57±0.92 a	14.72±0.43 a	36.72±1.92 a	19.35±1.22 a	37.17±2.62 a
I_SH		15.77±1.91 b	7.1±1.19 b	3.82±0.68 b	2.65±0.81 b	0.07±0.07 b	0±0 b
K_RHN		39.73±0.63 a	19.33±2.28 a	12.63±1.49 a	33.72±2.74 a	18.72±1.73 a	36.03±1.27 a
K_SH		16.57±0.26 b	8.13±0.31 b	4.95±0.38 b	2.58±0.3 b	0±0 b	0±0 b
H	F	1.06	1.36	0.30	0.79	0.11	0.15
	p val	*0.333*	*0.278*	*0.599*	*0.401*	*0.750*	*0.707*
W	F	329.58	101.46	114.99	355.54	320.91	631.70
	p val	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*
H:W	F	2.57	3.68	3.45	0.72	0.07	0.15
	p val	*0.148*	*0.091*	*0.101*	*0.421*	*0.796*	*0.707*

Note: Different letters indicate significant differences between treatments at each individual of all six assessments.
Legend: H - maize hybrid, W - water regime, RHN - normal hydric regime, SH - hydric stress, C - chlorophyll content 1-6- all six assessments, I - Inteligens maize hybrid, K - Kashmir maize hybrid.

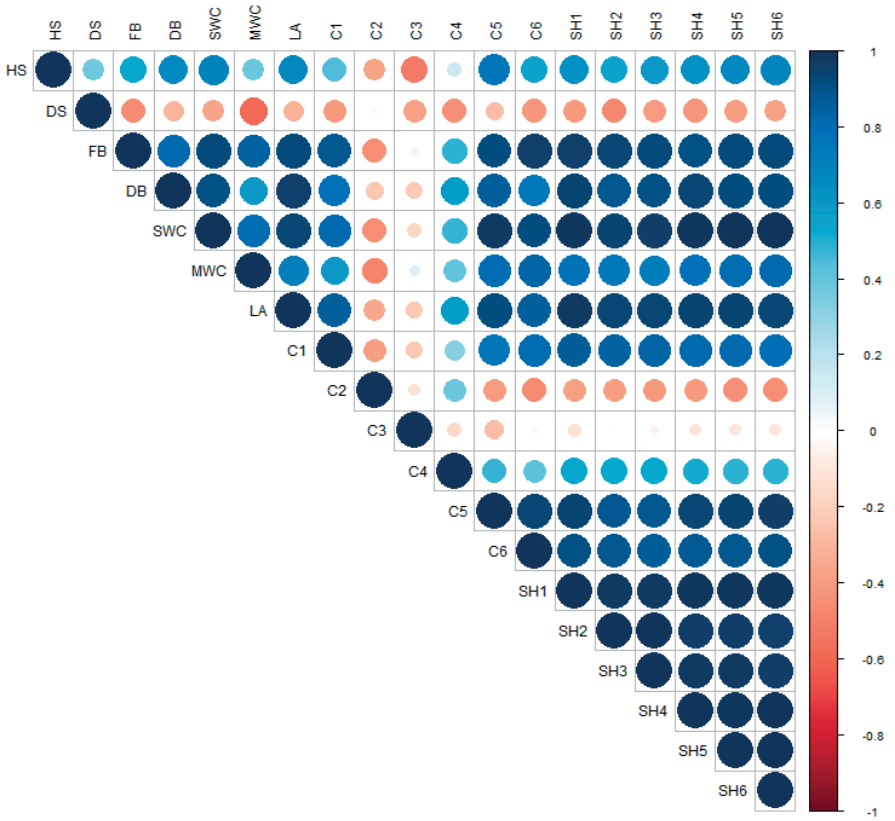


Figure 5. Correlogram chart between all analyzed parameters
Legend: The blue shades dots indicate positive correlations and the red shades dots indicate negative correlations between variables. HS, DS, FB, DB, SWC, MWC, LA, C1-C6, SH1-SH6

The correlation patterns between all registered and calculated values highlight both positive and negative effects with different strengths (Figure 5). Negative average correlation was observed between dry soil and fresh biomass, dry biomass, soil water content, maximum plant water content, leaf area, almost all assessments of chlorophyll content except the

second one and all assessments of soil humidity. The chlorophyll content from first assessment and most of the morphological features are negatively correlated with the second and third evaluation. Morphological parameters assessed at the end of the experiment correlate strong and positively with chlorophyll content from the assessment 5 and 6 and with all six assessments regarding soil humidity.

CONCLUSIONS

Both hybrids endured drought conditions for up to 23 days from emergence to wilting.
The Kashmir hybrid exhibited higher water requirements and achieved the highest chlorophyll content. In contrast, the Inteligens hybrid was more resilient to water stress, showing the most advanced development on the BBCH scale, the largest accumulated biomass, and the most extensive leaf area.
The highest chlorophyll content was recorded in KWS Kashmir under normal water regime.
The most resistant to water stress conditions (drought) was the KWS Inteligens hybrid according to assessment 3 and 4 in terms of development on the BBCH scale.
The maximum leaf area was recorded in the KWS Inteligens hybrid.
Dry biomass is directly correlated with soil moisture; a higher water requirement was observed in KWS Kashmir compared to KWS Inteligens.
The assessment of water stress in corn cultivation in Romania is essential for ensuring sustainable agricultural production and for protecting the country's food security.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

EFFECT OF FOLIAR FERTILIZATION ON PROTEIN LEVELS AND NUTRITIONAL VALUE OF THREE HYBRID MAIZE BIOMASS

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Abstract

Foliar fertilization is a widely used practice for correcting nutrient deficiencies in plants. The object of the present study are three corn hybrids (DKC 4416, LG 31.390 and Premeo), with different genetic origins. Variants of the field experience are 1. Control without irrigation, with soil fertilization with N14 in the form of ammonium nitrate; 2. Soil fertilization with N14 in the form of ammonium nitrate; 3. Fertilization with N14 + foliar fertilization with Aminosol +Lebozol B + Lebosol Zn, Nutriplant 36; 4. Fertilization with nitrogen N14 + Kinsidro Grow, N-Lock. In variants 2, 3 and 4, irrigation was carried out, with a drip irrigation system. The parameters of yield of green mass and yield of dry matter were determined. The content of crude protein, KEM, KER and PSC in green mass was calculated. Linear regression equations were developed, which show that there is a strong positive correlation between green mass yield and crude protein content. The correlation coefficients for the three hybrids r = 0.99 (DKC 4416), r = 0.96 (LG 31.390) - r = 0.93 (Premeo) were established.

Key words*: corn, green mass, foliar fertilization, nutritional value.*

INTRODUCTION

Practice has shown that one of the most powerful agrotechnical factors for increasing yields from a unit of area is fertilization (Mineva et al., 2016; Meng et al., 2018; Ray et al., 2019; Stoyanova et al., 2019a; Stoyanova et al., 2019b). That is why, in order to avoid unfavorable changes in the soil as a result of fertilization, approaches are sought for balanced and tailored to the specific requirements of each crop, variety and hybrid, with the phenophase of development and the level of applied agronomic plant nutrition. Climatic conditions, especially the length of the growing season directly affect the selection of maize hybrids (Zhao et al., 2017; Ileri et al., 2018; Bonea et al., 2019).

Green fodder is considered to be one of the most important factors for successful dairy farming. Maize is one of the most studied crops because of its high genetic diversity and is gaining increasing popularity and market demand, as grain, animal feed and other industrial purposes (López-Reyes et al., 2015). Maize is a valuable forage because it grows rapidly, forms rich biomass and helps increase body weight and milk quality in cattle due to its higher nutritional value (Iqbal et al., 2006; Chaudhary et al., 2016; Ferraretto et al., 2018).

According to Wadhwa et al. (2010) crop contained, in corn forage, the highest organic matter (OM) and crude protein (CP) content and the lowest neutral detergent fiber (NDF), acid detergent fiber (ADF), cellulose and hemicellulose content compared to other forages. In addition, maize harvested at milk maturity phenophase frees up areas for subsequent crops (Brar et al., 2017).

In recent years, selection has focused on the creation of high-yielding, ecologically plastic and high-nutritional forage corn hybrids (Hundal et al., 2019). The existing rich genetic diversity makes it possible to correctly select the appropriate hybrids (Keskin et al., 2017; Zaragoza et al., 2019). Newly created hybrids should show consistent performance and adaptability under different environmental conditions. (Nyirenda et al., 2021 opined).

The one-sided application of mineral fertilizers leads to a disturbance of the ecological balance and a decrease in the quality of production (Wei et al., 2020). The need to add micronutrients is a problem that has been recognized for a long time (Enakiev et al., 2018).

Foliar fertilization is a widely used practice to correct nutrient deficiencies in plants caused by improper or insufficient nutrient supply to the roots. Nutritional management influences crop performance. Adequate supply of nutrients during the growing season greatly affects the quantity and quality of yields (Subrahmanya et al., 2017; Lato et al., 2012; Niu et al., 2021).

As per Kumar et al. (2016) soil and foliar applied zinc sulfate had significant effects on dry matter yield and quality of maize forage. Djurdjic et al. (2017) reported an increase in yield, organic matter and crude protein after the combination of classical fertilization and foliar fertilization of maize with the organo-mineral fertilizer "Synergon 2000".

To increase plant height, leaf area index, fresh matter by more than 40%, and leaf area, Brankov et al. (2020), after foliar application. A positive effect on the accumulation of crude protein was established by Semina et al. (2020) after foliar application of the complex fertilizers with macroelements in a chelated form (Azosol 36 Extra and Aquarin 5).

Through foliar fertilizing, the nutritional regime of the plants is optimized and they can develop their productive potential. Breeders could help develop cultivars and hybrids that respond well to foliar feeding, Kannan (2010) believes.

The aim of the present study was to evaluate the energy and protein nutritional value of the biomass of three maize hybrids of different genetic origins, fed with foliar fertilizers during the growing season.

MATERIALS AND METHODS

To achieve the goal, a field experiment with three corn hybrids was conducted. The region in which the experiment was carried out belongs to the European-continental climate zone, the Stara Zagora region, Bulgaria. The object of the study are three corn hybrids: DKC 4416 (FAO 330), which is a representative of the FieldShield technology. The hybrids are characterized by high resistance to abiotic stress. LG 31.390 (FAO 390) is a representative of Limagrain's Hydraneo technology. These are a new generation of hybrids for drought risk management. Premeo is the representative of Syngenta's Artesian technology. The advantages of the technology are yield stability in the event of a deficiency of rainfall and soil moisture. The hybrid is moderately early with FAO 400.

During the vegetation period of the corn hybrids, foliar fertilization was carried out with fertilizers rich in macro and microelements. Foliar fertilization, with organic and inorganic foliar fertilizers, was carried out with the recommended doses for each of them. According to the first technology, including the organic liquid fertilizer Aminosol and the single-component fertilizers Boron, Zinc and Nutriplant (var. 3), fertilization was carried out in a dose of Aminosol (2.0 l/ha), Lebosol B (2.0 l/ha), Lebosol Zn (1.0 l/ha) and Nutriplant 36 (10.0 l/ha). According to the second technology (var. 4), the applied products are the organic fertilizer Kinsidro Grow (150 g/ha), in the form of granules, and the nitrogen stabilizer N-Lock (2.50 l/ha).

The experiment was conducted under irrigated and non-irrigated conditions. Variants of the study are as follows:1. Control - without irrigation, with soil fertilization with N14 in the form of ammonium nitrate; 2. Soil fertilization with N14 in the form of ammonium nitrate; 3. Fertilization with N14 + foliar fertilization with Aminosol +Lebozol B + Lebosol Zn, Nutriplant 36; 4. Fertilization with nitrogen N14 + Kinsidro Grow, N-Lock. In options 2, 3 and 4, irrigation was carried out, with a drip irrigation system, to maintain a sufficient amount of readily available moisture for the 0-50 cm soil layer.

By the formulas of Todorov et al. (2007) the FUM, FUG, PDI and RDP content for ruminants were calculated.

GE = 0 .0242 CP + 0.0366 EE + 0.0209 CF + 0.017 NFE

ME = 0.0152 DP + 0.0342 DEE + 0.0128 DCF + 0.0159 DNFE

q = ME / GE

FUM = ME (0.075 + 0.039q)

FUG = ME (0.04 + 0.1q)

PDI = 1.11CP (1 - Deg) Dsi + 0.093 FOM

FOM = DOM - DEE - FP - CP (1 - Deg)

FP = 250 – 0.5 DM

RDP = CP (Deg – 0.1) – 0.145 FOM

where: CF - crude fibre, CP - crude protein, DEE - digestible ether extract, Deg - degradability of dietary protein in the rumen, DF - digestible fibre, DNFE - digestible nitrogen free extract, DOM - digestible organic matter, DP - digestible protein, Dsi - digestibility in small intestine, EE

- ether extract, FOM - fermentable organic matter, FP - silage fermentable products, FUG - feed unit for growth (= 6 MJ net energy for growth), FUM - feed unit for milk (= 6 M J net energy for lactation), GE - gross energy, ME - metabolizable energy, NFE - nitrogen free extract, PDI - protein digestible in (small) intestine, RDP - rumen degradable protein.

RESULTS AND DISCUSSIONS

During the three farming years, the field experiment was based on the optimal sowing dates. During the crop's vegetation, biometric measurements and phenological observations were carried out. Corn hybrids with different genetic origins and with different resistance to environmental conditions have been tested in the field study, especially with regard to adaptability to soil moisture deficits. The results show the parameters of productivity in the three studied hybrids of corn (Table 1).

The values of the green mass presented in the table show that in conditions of natural moisture and supply, the yield of green mass varies over the years from 14000.0 kg (at DKC 4416) to 22300.0 kg (at LG 31.390). On average for the study period it was found that the hybrid DKC 4416 forms a green mass of 18100.00 kg/ha, followed by LG 31.390, with 19133.33 kg/ha. For the three-year period, under non-irrigated conditions, the highest yield of green mass was registered at Premeo - 20166.67 kg/ha. In the variants with irrigation, without foliar fertilization, an increase in the productivity of the hybrids was established.

When optimizing the moisture in the soil horizon, a tendency towards an increase in the obtained biomass was recorded for all hybrids. The increase in the productivity of the green mass was respectively 182.4% for Premeo, 19.2% for LG 31.390 and 216.3% for DKC 4416. The data analysis shows the parameters of the influence of readily available moisture in the soil. Yields increased to 57,254.8 kg/ha for DKC 4416, to 58,531.7 kg/ha for Premeo, and to 54,028.5 kg/ha for LG 31,390.

The treatment with foliar fertilizers in var. 3 and 4 leads to an increase in the productivity of maize hybrids at different rates over the years. In the first year after the application of the organic fertilizer Kinsidro Grow and the nitrogen stabilizer N-Lock, 100,675.7 kg/ha of green mass was registered at DKC 4416, and 101,976.5 kg/ha at LG 31.390, while the hybrid Premeo (102,320.9 kg/ha) was more responsive to foliar fertilization with the organic fertilizer Aminosol and the single-component fertilizers Boron, Zinc and NutriPlant. In the year 2023 a tendency to increase the yield of green mass after treatment with Aminosol, Boron, Zinc and Nutriplant (var. 3) was reported. An increase was also reported in var. 4, but with a smaller growth in yield. In the last year of the study, the trend towards increasing green mass yield values after applying foliar fertilization products was maintained, with var. 3, higher yield values were again registered.

On average for the three-year period, an increase of 29.8% (for Premeo), 38.6% (DKC 4416) to 48.3% (for LG 31.390) was found after application of the organic fertilizer Kinsidro Grow and N-Lock. 43.4% (at DKC 4416) and by 49.6% (at LG 31.390). The results indicate that the improvement of the nutritional regime during the vegetation of the crop helps to increase the green mass yield.

Optimizing the water and food regimes create conditions for unfolding the productive potential of the culture. In addition to yields, humidity and nutritional conditions also affect the parameters of dry matter and crude protein content. Figure 1 shows how the values of crude protein increase after irrigation to optimize the moisture factor, as well as after the application of foliar fertilizers rich in macro and microelements. The diagram shows the positive trend towards an increase in crude protein content in the green mass. The lowest values of the indicator were found at var. 1.

In the three studied hybrids, an increase in crude protein content under the influence of the artificially applied irrigation water was reported. In DKC 4416, the increase in the values of the indicator was 182.3% compared to the non-irrigated variant, on average for the period of the study. The LG 31.390 hybrid responded with a 109.7% increase and Premeo with 98.6% after optimizing the soil moisture.

Table 1. Energy and protein nutritional content of the green mas

Hybrids of maize	Variants	Green matter, kg	DM, kg	FUM	FUG	CP, kg	PDI, kg
2022							
DKC 4416	1	14000,00	6171,55	6917,13	7201,50	501,15	537,80
	2	43466,70	15730,69	18306,70	19179,40	1347,86	1400,85
	3	57267,00	21140,28	24314,26	25429,42	1888,51	1893,80
	4	49133,30	17458,97	19411,26	20214,52	1538,95	1524,50
LG 31.390	1	16400,00	7607,96	8434,45	8707,92	701,45	685,34
	2	37133,00	12918,88	14524,94	15111,31	1182,08	1154,72
	3	56033,00	19333,47	21478,88	22362,13	1869,55	1743,48
	4	53633,00	18758,25	21607,71	22596,86	1800,79	1718,20
Premeo	1	17200,00	6710,57	7358,01	7594,80	614,20	599,11
	2	39866,70	13863,41	16094,65	16845,85	1296,53	1263,67
	3	48273,30	16942,63	19584,09	20448,48	1681,11	1574,49
	4	43533,30	14773,88	16115,58	16693,95	1509,23	1346,36
2023							
DKC 4416	1	21800,00	7303,59	7925,76	8193,13	687,14	639,92
	2	72648,90	15857,25	16723,21	17104,28	1972,04	1506,90
	3	100545,70	22602,46	23672,67	24159,29	2763,22	2133,70
	4	100675,70	22668,72	24076,96	24678,70	2669,19	2109,81
LG 31.390	1	22300,00	7959,35	8729,23	9058,88	682,91	686,11
	2	70978,40	15353,40	16455,17	16900,63	1578,33	1386,79
	3	101137,50	25894,18	28305,07	29243,90	2703,35	2354,88
	4	101976,50	24198,50	26016,49	26814,89	2415,01	2159,91
Premeo	1	24100,00	8245,54	8848,78	9116,39	705,71	704,10
	2	74774,20	16256,70	17266,86	17740,16	1416,27	1398,84
	3	102320,90	23559,69	24755,08	25193,89	2681,57	2179,97
	4	99668,30	20599,93	21729,10	22277,03	2228,81	1859,61
2024							
DKC 4416	1	18500,00	5868,89	6271,50	6427,03	530,45	511,48
	2	55648,90	14852,66	16265,05	16820,41	1532,16	1346,75
	3	88455,40	21977,00	24097,06	24897,54	2472,29	2040,15
	4	88172,90	21118,26	23165,92	23919,70	2302,73	1949,19
LG 31.390	1	18700,00	5853,23	6166,95	6301,07	587,78	522,56
	2	53974,20	12597,30	13530,19	13886,81	1374,67	1158,08
	3	85312,00	21788,58	23347,21	23984,99	2423,81	2006,82
	4	84682,30	22727,18	25035,66	25918,21	2242,38	2046,73
Premeo	1	19200,00	6633,19	7040,15	7186,97	822,62	630,36
	2	60954,30	13803,57	15295,27	15786,25	1541,48	1285,04
	3	86826,00	23016,06	25461,91	26294,93	2346,80	2087,77
	4	84785,50	17695,53	18931,84	19367,11	1941,99	1633,43
Average 2022-2024							
DKC 4416	1	18100,00	64489,01	7038,13	7273,89	572,91	563,07
	2	57254,83	15480,20	17098,32	17701,36	1617,35	1418,17
	3	82089,37	21906,58	24028,00	24828,75	2374,67	2022,55
	4	79327,30	20415,32	22218,05	22937,64	2170,29	1861,17
LG 31.390	1	19133,33	7140,18	7776,88	8022,62	657,38	631,34
	2	54028,53	13623,19	14836,77	15299,58	1378,36	1233,20
	3	80827,50	22338,74	24377,05	25197,01	2332,24	2035,06
	4	80097,27	21894,64	24219,95	25109,99	2152,73	1974,95
Premeo	1	20166,67	7196,43	7748,98	7966,05	714,18	644,52
	2	58531,73	14641,23	16218,93	16790,75	1418,09	1315,85
	3	79140,07	21172,79	23267,03	23979,10	2236,49	1947,41
	4	75995,70	17689,78	18925,51	19446,03	1893,34	1613,13

Feeding with foliar fertilizers leads to an increase in the crude protein index values. After treatment with the products Aminosol, Boron, Zinc and Nutriplant, an increase of 213.2%, 254.8% and 314.5% was found, respectively, in the hybrids Premeo, LG 31.390 and DKC 4416. The increase in the index after fertilization with Kinsidro Grow and N-Lock was 165.1%, 227.5% and 278.8%, respectively, in Premeo, LG 31.390 and DKC 4416.

The increase in the index after fertilization with Kinsidro Grow and N-Lock was 165.1%, 227.5% and 278.8%, respectively, in Premeo, LG 31.390 and DKC 4416. The maize hybrid DKC 4416 is the most responsive to the optimized feeding regime.

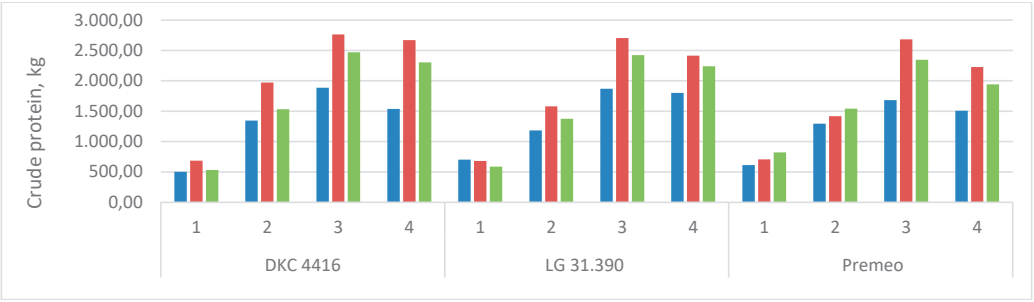


Figure 1. Content of crude protein (kg), by hybrids maize and years

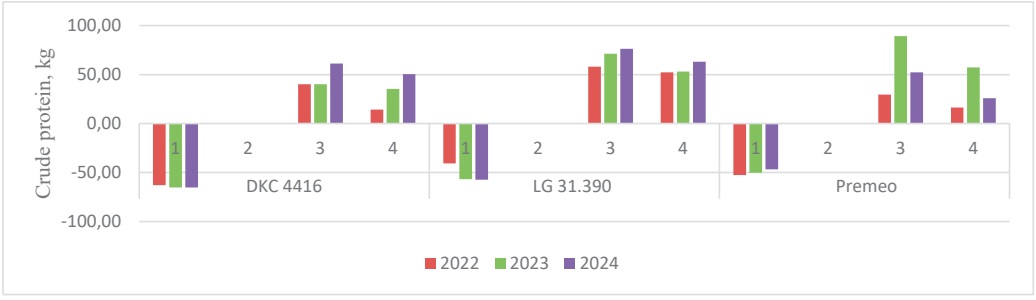


Figure 2. Content of crude protein (kg), compared to var. 2, by hybrids maize and years

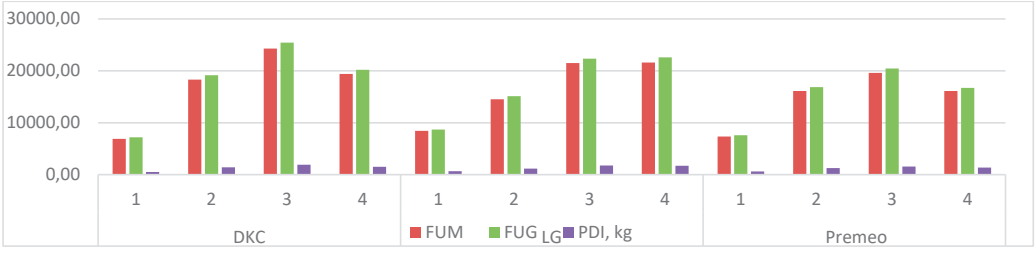


Figure 3. Content of FUM, FUG and PDI in kg, by hybrids and variants, on average for the 2022-2024 period

If, however, the data from var. 2 are accepted as control and a comparison was made with the results of the other options, it can be seen that when soil nutrition is implemented, but without irrigation, how the crude protein content in the green mass decreases. The reduction in crude protein was 49.6% (at Premeo), 52.3% (at LG 31.390) and by 64.6% (at LG 31.390). On the other hand, taking var. 2 for the control, the values of the indicator immediately stand out, increased thanks to the macro and micro elements brought in during the vegetation, through the foliar fertilizers (Figure 2). The increase in crude protein content under the influence of foliar fertilization was 46.8%, 57.7% and 69.2%, respectively, for DKC 4416, Premeo and LG 31.390, and was under the influence of feeding with the organic fertilizer Aminosol and the monocomponent Boron, Zinc and Nutriplant. The treatment with the organic fertilizer Kinsidro Grow and the nitrogen stimulator N-Lock contributed to an increase in the content of the indicator by 33.5%, 34.2% and 56.2%, respectively, in Premeo, DKC 4416 and LG 31.390.

The energy and protein nutritional content of the green mass was calculated.

The parameters of FUM, FUG and PDI were established for hybrids and variants. Figure 3 shows the positive trend towards increasing values of milk feed units (FUM) in lactating animals, feed growth units (FUG) in growing animals and digestible protein in the small intestine of ruminants PDI. The positive trend towards growth with optimization of conditions – moisture and nutrients, confirms the influence of both factors in the three corn hybrids. Higher values were calculated for DKC 4416, followed by LG 31.390 and Premeo.

The conducted regression analysis establishes a high positive correlation between green mass yield and the content of crude protein, in kg per ha. The correlation coefficients for the corn hybrids studied were established – r = 0.99 (DKC 4416), r = 0.96 (LG 31.390) – r = 0.93 (Premeo). Linear regression models were developed and the coefficients of determination determined. R2 = 0.8733 Premeo, R2 = 0.929 LG 31.390, R2 = 0.9823 at DKC 4416 (Figure 4).

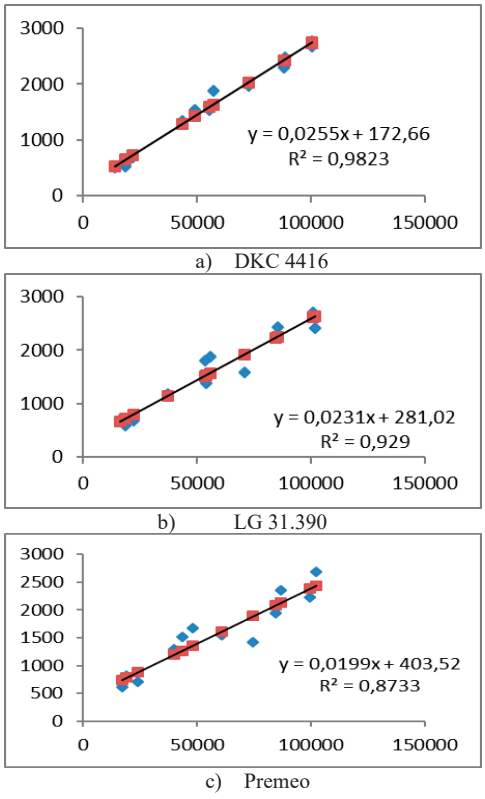


Figure 4. Linear regression models between yield of green mass and the content of crude protein, in kg/ha, at a) DKC 4416, b) LG 31.390 and c) Premeo

The coefficient of determination indicates the percentages of variation in crude protein content, i.e. they express the influence of green mass yield on the crude protein content of that mass.

CONCLUSIONS

As a result of the conducted field study, several main conclusions can be drawn:

Fertilization with foliar fertilizers leads to an increase in the values of the crude protein indicator. After treatment with the products Aminosol, Boron, Zinc and Nutriplant, an increase of 213.2%, 254.8% and 314.5% was found, respectively, in the hybrids Premeo, LG 31.390 and DKC 4416. The increase in the index after fertilization with Kinsidro Grow and N-Lock was 165.1%, 227.5% and 278.8%, respectively, in Premeo, LG 31.390 & DKC 4416.

A positive trend towards an increase in FUM, FUG and PDI indicators was established with the optimization of the water and nutrition regime of hybrid corn.

A high positive correlation between green mass yield and crude protein content was found, correlation coefficients r = 0.99 (DKC 4416), r = 0.96 (LG 31.390) – r = 0.93 (Premeo).

Linear regression models were developed and the coefficients of determination were determined for the three corn hybrids - $R^2 = 0.87$ (Premeo), $R^2 = 0.93$ (LG 31.390), $R^2 = 0.98$ for DKC 4416.

ACKNOWLEDGEMENTS

This research work was funded by Bulgarian Ministry of Education and Science (MES) in the frames of Bulgarian National Recovery and Resilience Plan, Component "Innovative Bulgaria", the Project № BG-RRP-2.004-0006-C02 “Development of research and innovation at Trakia University in service of health and sustainable well-being and project 1AF/23, with topic: “Agrobiological evaluation of the yield and quality indicators of maize for silage and for grain from introduced hybrid maize”.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

STUDIES ON THE CULTIVATION OF WHITE MUSTARD (*Sinapis alba*) UNDER THE AGROECOLOGICAL CONDITIONS OF SOUTHERN DOBROGEA – DELENI IN A CONVENTIONAL SYSTEM

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Abstract

This study explores the behavior of white mustard (Sinapis alba) varieties under the specific agroecological conditions of southern Dobrogea. The experiments were conducted in the locality of Deleni, Constanța County, on two indigenous varieties, Bella and Cezara. The same agricultural technology was applied to both varieties, with the main objectives being to determine the optimal sowing density, analyze plant density at emergence and harvest, and assess the qualitative indicators of production. The results indicate that both varieties exhibit significant adaptive characteristics to the agroecological context of the region, yielding favorable average production levels in the cultivation years of 2022, 2023, and 2024. Additionally, the findings highlight the impact of environmental factors such as precipitation levels and soil conditions on plant development and productivity. The mean and standard deviation were calculated for each crop variant (Cezara and Bella, with and without fertilizer) both at emergence and at harvest, and an ANOVA test was applied to check whether the differences between variants were statistically significant. Additionally, a student's t-test was used to compare the means between the fertilized and control variants for each variety. The results showed that the differences between variants were statistically significant ($p < 0.05$), indicating a clear effect of fertilization on the increase in the number of siliques. To verify the normality of the data distribution, the Shapiro-Wilk test was used. p-values greater than 0.05 suggest that the distribution of the number of siliques per plant follows a normal distribution, validating the applicability of the parametric tests used. A regression analysis was conducted to assess the impact of plant density on yield. Linear regressions indicated a positive correlation between plant density at harvest and the obtained yield, with significant determination coefficients (R^2). These results contribute to a better understanding of the potential of Sinapis alba in conventional farming systems and offer insights into optimizing cultivation techniques for sustainable agricultural production in this region.

Key words: *mustard, climatic factors, cultivation, fertilizer, production, Southern Dobogea.*

INTRODUCTION

The cultivation of white mustard (*Sinapis alba*) is particularly important due to its multiple uses, ranging from its phytosanitary role to its application in the food industry (Popescu & Ionescu, 2019). This plant is widely recognized for its ability to suppress soil-borne pathogens, improve soil structure, and act as a natural biofumigant, making it an essential component in sustainable agriculture (Gheorghe et al., 2022; Risnoveanu et al., 2022). Additionally, white mustard contributes to weed suppression and enhances soil fertility by increasing organic matter content and nutrient cycling (Smith & Thompson, 2017; Titei, 2022). The effectiveness of different mineral fertilization strategies has been analyzed in relation to mustard cultivation, highlighting its role in maintaining soil productivity (Suvet et al., 2024).

In Romania, this crop has been promoted as a solution for diversifying crop rotation, considering its drought resistance, high adaptability to different soil types, and short vegetation period, which allows for flexibility in planting schedules (Axinte et al., 2006; Panaitescu, 2008; Panaitescu & Niță, 2011). The adaptability of various agricultural plants to dry-farming systems in southeastern Romania has been studied, emphasizing the potential of mustard as a resilient crop (Risnoveanu et al., 2022). White mustard is also valuable due to its potential applications in biofuel production, green manure, and livestock feed, given its high protein and essential oil

content (Titei, 2022). Moreover, the role of innovative seed treatments in improving germination and seedling vigor has been explored, further supporting its agronomic importance (Epure et al., 2024). Deleni, the locality where the studies were conducted, has a temperate-continental climate influenced by its proximity to the Black Sea coast, with an average annual temperature of 11.5°C and average annual precipitation below 400 mm (Ministry of Agriculture and Rural Development, 2023). This region is characterized by frequent periods of water deficit, requiring crops with strong drought tolerance to ensure stable yields. The predominant soils are chernozems, known for their high humus content, good moisture retention, and natural fertility, making them highly suitable for agricultural production (Panaitescu, 2016). Given these conditions, the selection of well-adapted mustard varieties plays a crucial role in maximizing yield potential and ensuring economic sustainability (Iosub et al., 2022).

White mustard cultivation integrates well into local agricultural systems due to its low input requirements, fast germination rate, and ability to thrive in soils with variable fertility levels (Smith & Thompson, 2017). Furthermore, its deep root system helps prevent soil erosion, improves nutrient cycling, and enhances organic matter content, contributing to long-term soil health (Jia et al., 2020). The effectiveness of different treatments in controlling crop diseases has been examined, with implications for integrated pest management strategies in mustard cultivation (Iosub et al., 2022; Toader et al., 2024). The use of thyme essential oils has also been studied for their antifungal properties, which could serve as an alternative seed treatment in sustainable mustard farming (Gheorghe et al., 2022). Recent studies have highlighted the impact of plant growth regulators on seedling morphology, further demonstrating the agronomic potential of white mustard under controlled conditions (Jia et al., 2020). Additionally, research into its fresh and ensiled biomass quality suggests promising applications in animal nutrition and fodder production (Titei, 2022). The influence of sustainable agricultural practices, including reduced pesticide use and eco-friendly fertilization techniques, is increasingly relevant in the context of climate change and environmental conservation (Toader et al., 2024).

In this context, the present study aims to analyze the agronomic performance, yield potential, and adaptability of two Romanian white mustard varieties, Bella and Cezara, cultivated under the specific conditions of southern Dobrogea. The research evaluates key parameters such as emergence density, plant development, biomass accumulation, and seed productivity, providing valuable insights into optimizing mustard cultivation in this region. The results contribute to the development of the best management practices that enhance both economic profitability and ecological sustainability in conventional farming systems.

MATERIALS AND METHODS

The experiments were conducted in Deleni, Constanța County, under the climatic conditions characteristic of southern Dobrogea. The region is known for its temperate-continental climate, influenced by its proximity to the Black Sea, which results in moderate winters and hot, dry summers. The soil in the area are predominantly chernozems, rich in humus and naturally high in fertility, making them well-suited for agricultural production. The average annual temperature is 11.5°C, while the average annual precipitation remains below 400 mm, creating a challenging environment for crop cultivation and necessitating the selection of drought-resistant varieties.

The selected varieties for this study were Bella and Cezara, both of which are included in the Official Catalogue of Plant Varieties of Romania - 2022. Cezara is a semi-late Romanian mustard variety, known for its good adaptability to arid conditions and high yield stability. Bella, also a Romanian variety, is particularly valued for its antinematode properties, making it an excellent rotation crop to improve soil health. Like Cezara, it demonstrates strong drought resistance and is classified as a semi-late variety. For each variety, Bella and Cezara, two hectares were cultivated using the same agricultural

technology, alongside two control hectares for each variety, ensuring a comparative analysis of different cultivation approaches. In line with the study's goal, the following key objectives were established: Identifying an optimal cultivation technology variant that ensures high productivity and sustainability. Monitoring crop development throughout the entire vegetation period, with frequent field observations to assess plant health and growth stages. Determining key yield indicators and quality parameters of the harvested crop, including seed weight, biomass accumulation, and oil content. Additionally, the study aims to evaluate the response of these mustard varieties to the specific climatic and soil conditions of the region, providing scientific insights into their adaptability, resilience, and agronomic performance. Given the increasing concerns about climate change and soil degradation, understanding the role of mustard in sustainable agricultural systems is becoming increasingly important. By conducting a detailed analysis of plant density at emergence and harvest, seed yield, and overall productivity, this research contributes to the optimization of mustard cultivation practices in southern Dobrogea, a region where water scarcity and extreme weather events pose significant challenges to farmers.

The mean and standard deviation were calculated for each crop variant (Cezara and Bella, with and without fertilizer) both at emergence and at harvest, and an ANOVA test was applied to check whether the differences between variants were statistically significant. Additionally, a student's t-test was used to compare the means between the fertilized and control variants for each variety. The results showed that the differences between variants were statistically significant ($p < 0.05$), indicating a clear effect of fertilization on the increase in the number of siliques. To verify the normality of the data distribution, the Shapiro-Wilk test was used. p-values greater than 0.05 suggest that the distribution of the number of siliques per plant follows a normal distribution, validating the applicability of the parametric tests used. A regression analysis was conducted to assess the impact of plant density on yield. Linear regressions indicated a positive correlation between plant density at harvest and the obtained yield, with significant determination coefficients (R^2).

For each variety, Bella and Cezara, two hectares were cultivated using the same agricultural technology, along with an additional two hectares of control plots for each variety. Soil preparation included stubble breaking, autumn plowing, fertilization with complex NPK 15:15:15 fertilizers (200 kg/ha), and mechanized harvesting at full maturity when the grain moisture was below 16%. Sowing was carried out at a depth of 3 cm, with a row spacing of 25 cm and a sowing density of 120 viable seeds/m². The mustard was sown in the first urgency window each year of cultivation.

A microgranulated starter fertilizer, Microstar Care PZ NG (30 kg/ha), was also applied at sowing. This product combines essential nutrients and enhances root development and nutrient uptake, ensuring optimal early plant growth.

Crop monitoring included regular field observations, determination of plant density at emergence and harvest, and analysis of productivity indicators. Winter wheat was used as a preceding crop in all experiments. Standard agronomic maintenance practices were applied each year, including both preventive and curative measures. Weed control was carried out using Galera herbicide at a rate of 300 ml/ha in 300 liters of water. The product was applied when weeds were in their early developmental stages. After herbicide application, an additional treatment with the Alga 600™ biostimulator was performed. This product, derived from marine algae (*Sargassum* spp.), was applied at 1.5 kg/ha during the stem elongation phase to enhance plant vigor. Pest control was managed using Apis 200 SE at 250 ml/ha in 300 liters of water. Disease control was carried out using Amistar Gold fungicide at a rate of 1 liter/ha. In the control plots, neither the starter fertilizer nor the biostimulator was applied; only complex fertilizers were used. Otherwise, all other maintenance operations were identical across treatments.

Sowing followed variety-specific recommendations, maintaining a density of 120 viable seeds per m². The emergence density was assessed based on counts taken over 10 linear meters on two adjacent rows, in three

repetitions along the field diagonal over the three years of study.

RESULTS AND DISCUSSIONS

If 300 viable seeds were sown per 10 linear meters, the resulting plant densities were recorded as follows: for the Cezara variety with fertilizer application, the average emergence density over three years was 84.77 plants/m², with 87.86 plants/m² in 2022, and a decline to 81.13 plants/m² in 2024 due to lower spring precipitation levels. The control variant (Cezara without fertilizer) had an average density of 76.44 plants/m², with 79.53 plants/m² in 2022, dropping to 70.26 plants/m² in 2024. A similar trend was observed for the Bella variety, with 2024 experiencing a notable decline in emergence density due to drought conditions. The three-year average emergence density for fertilized Bella was 81.30 plants/m², while the control variant had 78.19 plants/m², more data can be observed in Table 1. The data indicate a clear decrease in emergence density in 2024, primarily due to lower precipitation levels. Fertilized variants consistently demonstrated higher emergence densities compared to control plots. The application of fertilizers and biostimulators significantly improved plant establishment, highlighting their importance in mitigating the adverse effects of drought conditions.

Fertilized variants consistently demonstrated higher emergence densities compared to control plots. This underscores the role of nutrient supplementation in promoting early plant vigor, particularly under water-deficient conditions. Biostimulators and fertilizers significantly improved plant establishment, supporting: enhanced seedling vigor, leading to better survival rates, improved root system development, which may have contributed to greater resilience to soil moisture fluctuations, stronger plant growth, ensuring better competition for resources in early vegetative stages.

The data indicate a clear decrease in emergence density in 2024**,** primarily due to lower precipitation levels. The analysis suggests that years with reduced spring rainfall had a direct impact on early plant establishment, emphasizing the importance of water availability during germination and emergence stages**.**

The harvest density was determined using similar methods, resulting in an average of 66.84 plants/m² for the Cezara mustard variant with fertilizer**,** compared to only 62.26 plants/m² for the control variant**.** For the Bella variant with fertilizer**,** the three-year average density was 61.35 plants/m²**,** whereas the control variant had only 56.22 plants/m²**,** as shown in Table 2**.** The reduction in density can be attributed to factors such as soil dryness during the early vegetation period and pest attacks.

Based on these determinations, it can be stated that major importance should be given to several factors and indicators: optimizing the sowing density to 70-72 plants/m² to maximize yield, using fertilizers and biostimulants to improve plant vigor and production, closely monitoring climatic conditions, and adapting cultivation technologies to drought, especially since prolonged droughts and an increase in the average annual temperature have been recorded in the study area in recent years.

The mean and standard deviation were calculated for each crop variant (Cezara and Bella, with and without fertilizer) both at emergence and at harvest, the data can be found in Figure 1. The data indicate that the fertilized variants tend to have a higher density compared to the control variants. An ANOVA test was applied to check whether the differences between the variants are statistically significant. Emergence density: F-statistic = 1.29, P-value = 0.3431. There are no significant differences between the variants at emergence. Harvest density: F-statistic = 2.25, P-value = 0.1598. There are no significant differences between the variants at harvest. To assess the stability of density values at emergence and harvest over three years, the standard deviation was calculated: emergence density: Cezara fertilized: 3.393, Cezara control: 5.3520, Bella fertilized: 5.3425, Bella control: 7.5104. Harvest density: Cezara fertilized: 6.7464, Cezara control: 5.7694, Bella fertilized: 3.4504, Bella control: 3.3354, more date can be found in Table 3. Higher standard deviation values indicate greater variation in data from year to year. The higher standard deviation for fertilized Cezara suggests that this variant was

more sensitive to climatic changes. Conversely, Bella had a lower standard deviation, indicating greater stability over time, even under more challenging conditions. Cezara appears to respond more strongly to variable climatic conditions, while Bella is more stable. However, in both cases, the application of fertilizer and biostimulant contributes to higher density at both emergence and harvest.

The determination of the number of siliques per plant was carried out in the field by selecting a representative sample of five plants along the diagonal of the field. Measurements were conducted in three repetitions along the diagonal for each variant in each cultivation year. The results of the counts are presented in the table below.

Table 1. Average emergence density of Cezara and Bella mustard varieties in control and fertilized variants with starter fertilizer and biostimulator

Year	Variety/ variant	Row 1 Plant count	Row 2 Plant count	Average per replication Plant count per row (10 linear m)	Density per square meter (plants/m²)
2022	Cezara /V1	217.33	222	219.66	87.86
	Cezara control/ V2	202.33	195.33	198.83	79.53
2023	V1	215.33	211.33	213.33	85.33
	V2	199	198.66	198.83	79.53
2024	V1	201.66	204	202.83	81.13
	V2	177.66	173.66	175.66	70.26
Average over III years	**V1**	**211.44**	**212.44**	**211.94**	**84.77**
	V2	**192.99**	**189.21**	**191.1**	**76.44**
2022	Bella /V3	212.33	213	212.66	85.06
	Bella control/V4	212.33	210.66	211.5	84.6
2023	V3	209.67	208.67	209.17	83.67
	V4	201.33	199	200.16	80.06
2024	V3	190.33	185.66	187.99	75.19
	V4	174	175.66	174.83	69.93
Average over III years	**V3**	**204.11**	**202.44**	**203.27**	**81.30**
	V4	**195.88**	**195.10**	**195.49**	**78.19**

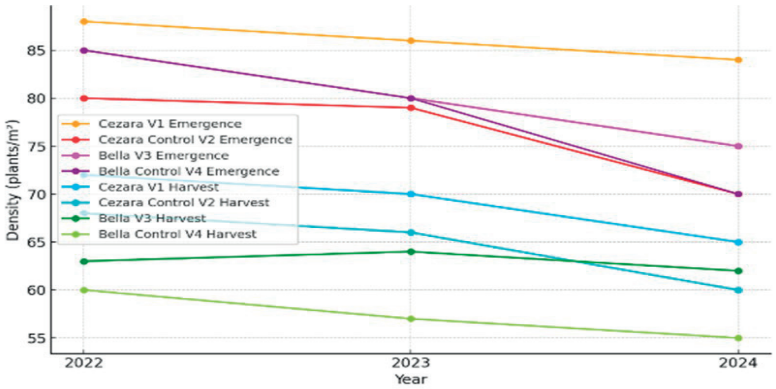


Figure 1. Evolution of density at emergence and harvest for the two mustard varieties

Table 2. Average Harvest Density of Cezara and Bella Mustard Varieties in Control and Fertilized Variants with Starter Fertilizer and Biostimulator

Year	Variety/ variant	Row 1 Plant count	Row 2 Plant count	Average per replication Plant count per row (10 linear m)	Density per square meter (plants/m²)
2022	Cezara /V1	179	179.33	179.16	71.66
	Cezara control/ V2	167	165.66	166.33	66.53
2023	V1	175.66	173	174.33	69.73
	V2	162	163	162.5	65
2024	V1	148.33	147.33	147.83	59.13
	V2	140.33	139	139.66	55.86
Average over III years	**V1**	**167.66**	**166.55**	**167.10**	**66.84**
	V2	**156.44**	**155.88**	**156.16**	**62.26**
2022	Bella /V3	154.33	159.66	156.99	62.79
	Bella control/V4	149.33	149	149.16	59.66
2023	V3	159	159	159	63.6
	V4	141.67	138.33	140	56
2024	V3	146.66	141.66	144.16	57.26
	V4	132.33	132.66	132.5	53
Average over III years	**V3**	**153.33**	**153.44**	**153.38**	**61.35**
	V4	**141.11**	**139.99**	**140.55**	**56.22**

Table 3 Statistical summary

Plant density statistical summary		
Variant	**Mean**	**Standard deviation**
Cezara V1 emergence	84.77	3.3993
Cezara control V2 emergence	76.44	5.3520
Bella V3 emergence	81.30	5.3425
Bella control V4 emergence	78.19	7.5104
Cezara V1 harvest	66.83	6.7464
Cezara control V2 harvest	62.46	5.7694
Bella V3 harvest	61.21	3.4504
Bella control V4 harvest	56.22	3.3354

For the Cezara variety with fertilizer application, the average number of siliques per plant over the three years of experimentation ranged between 130.2 and 131, with an overall mean of 130.53 siliques per plant. In contrast, for the control variant, the average number of siliques per plant over the three years varied between 117.8 and 118.46, with a three-year mean of 118.13 siliques per plant. For the Bella variety with fertilizer application, the average number of siliques per plant over the three years of experimentation ranged between 116.26 and 118.2, with an overall mean of 117.37 siliques per plant. In contrast, for the control variant, the three-year average ranged between 109.26 and 111.26, with a mean of 110.10 siliques per plant, data found in Table 4. These parameters highlight the high productive potential of these varieties despite climatic variability during the study years. Additionally, they emphasize the higher productive potential of the Cezara variety compared to the Bella variety.

Table 4. The average number of siliques per plant based on variety and variant

Year	Variety/ variant	Plants	Replication No. 1 Number of siliques per plant	Replication No. 2 Number of siliques per plant	Replication No. 3 Number of siliques per plant	Mean
2022	Cezara /V1	5	143.2	142.6	143.2	143
	Cezara control/ V2	5	134.4	133.6	133.2	133.73
2023	V1	5	132.4	131.2	133.6	132.4
	V2	5	120.6	118.8	119.6	119.66
2024	V1	5	117.4	116.8	114.4	116.2
	V2	5	98.4	103	101.6	101
Average over III years	**V1**	**5**	**131**	**130.2**	**130.4**	**130.53**
	V2	**5**	**117.8**	**118.46**	**118.13**	**118.13**
2022	Bella /V3	5	126.2	124.8	125.6	125.53
	Bella control/V4	5	121	120.2	124	121.73
2023	V3	5	123.2	120.4	121.8	121.8
	V4	5	120.4	118	119.4	119.26
2024	V3	5	123.2	120.4	121.8	121.8
	V4	5	88	89.6	90.4	89.33
Average over III years	**V3**	**5**	**117.66**	**116.26**	**118.2**	**117.37**
	V4	**5**	**109.8**	**109.26**	**111.26**	**110.10**

A regression analysis was conducted to evaluate the impact of plant density on yield (Figure 2).

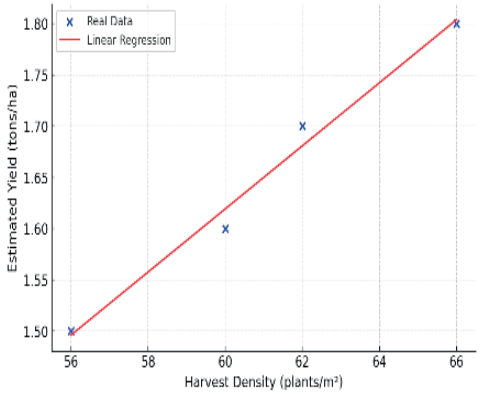


Figure 2. Graphical representation of the regression between harvest density and estimated yield

The linear regressions indicated a positive correlation between plant density at harvest and the obtained yield, with significant determination coefficients (R^2). The determination coefficient (R^2) = 0.944, meaning that 94.4% of the yield variation is explained by plant density at harvest. In other words, there is a very strong relationship between these two factors.

The regression coefficient (x1) = 0.0288, indicating that for every increase of 1 plant/m² in density at harvest, the estimated yield increases by 0.0288 tons/ha. The intercept (const) = -0.1264, which represents the point where the regression line intersects the yield axis. While it does not have a direct practical interpretation, it is necessary for the mathematical model. The p-value for the coefficient (P>|t|) = 0.028, which is below the threshold of 0.05, indicating a statistically significant relationship between the variables. The ANOVA results indicate a significant difference between the variants ($p < 0.05$), confirming the influence of fertilization on the number of siliques per plant. Additionally, a t-test (Student's t-test) was applied to compare the means between fertilized and control variants for each variety. The results showed that the differences between variants are statistically significant ($p < 0.05$), demonstrating a clear effect of fertilization on the increase in the number of siliques. To verify the normality of the data distribution, the Shapiro-Wilk test was used. The p-values > 0.05 suggest that the distribution of siliques per plant follows a normal distribution, validating the applicability of parametric tests. The coefficient of variation (CV%) was calculated

for each variant, highlighting the variability of the data. The obtained values fall within an acceptable range (<10%), confirming the accuracy of the determinations and the reproducibility of the measurements. Thus, the higher the plant density at harvest, the higher the estimated yield.

The average grain weight per silique was 0.0177 g. according to Table 5, while the thousand grain weight (TGW) ranged between 5.23 g and 5.31 g. These results presented in Figures 3 and 4, are consistent with previous reports for this variety, confirming its genetic qualities and adaptability under moderate drought conditions. Furthermore, the low variability in TGW values suggests stable grain development, even under fluctuating environmental conditions. This indicates a good balance between plant resource allocation and grain filling efficiency, contributing to overall yield stability.

Table 5. The average grain weight per silique per plant based on variety and variant

Year	Variety/ variant	Plants	Replication No. 1 grain weight per silique, g	Replication No. 2 grain weight per silique, g	Replication No. 3 grain weight per silique, g	Mean
2022	**Cezara /V1**	10	0.0219	0.0215	0.0214	0.0216
	Cezara control/ V2	10	0.0197	0.0196	0.0197	0.01966
2023	**V1**	10	0.0191	0.0188	0.0187	0.0188
	V2	10	0.0164	0.0159	0.0153	0.01586
2024	**V1**	10	0.0167	0.0163	0.0171	0.0167
	V2	10	0.0132	0.0122	0.0128	0.01273
Average over 3 years	**V1**	**10**	**0.0192**	**0.0188**	**0.0190**	**0.0190**
	V2	**10**	**0.0164**	**0.159**	**0.0159**	**0.01606**
2022	**Bella /V3**	10	0.0221	0.0215	0.0212	0.0216
	Bella control/V4	10	0.0185	0.0184	0.0187	0.01853
2023	**V3**	10	0.0184	0.0186	0.0184	0.01846
	V4	10	0.0161	0.0156	0.0152	0.01563
2024	**V3**	10	0.0164	0.0162	0.0161	0.01623
	V4	10	0.0129	0.0124	0.0122	0.0125
Average over III years	**V3**	**10**	**0.0189**	**0.0187**	**0.0185**	**0.0187**
	V4	**10**	**0.0158**	**0.0154**	**0.0153**	**0.0155**

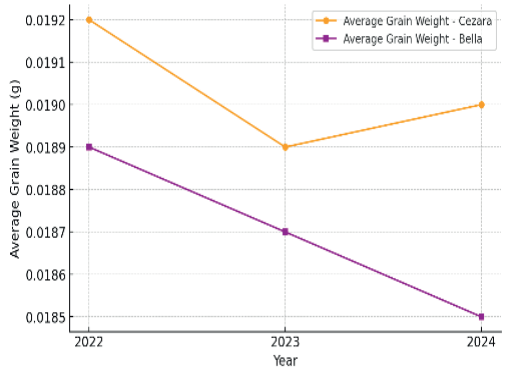


Figure 3. Evolution of the average grain weight of white mustard

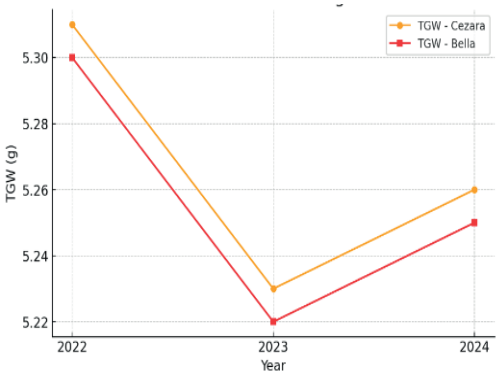


Figure 4. Evolution of the thousand grain weight of white mustard

The calculation of probable yield represented in Figure 5 highlighted an estimated productivity of approximately 1.8 tons/ha for the Cezara variety. This value aligns with the genetic potential of the variety and the agroclimatic conditions of the study year. Additionally, the stability of this yield suggests an efficient resource utilization and a good adaptation of the variety to environmental factors. The results confirm that Cezara maintains a high production potential, making it a reliable choice for cultivation under similar conditions.

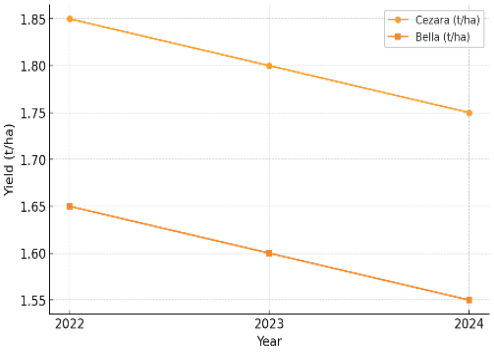


Figure 5. Evolution of White Mustard Yield

CONCLUSIONS

The study demonstrated that the Bella and Cezara varieties adapt efficiently to the agroecological conditions of southern Dobrogea. The Cezara variety exhibited higher productivity compared to Bella, confirming its status as a climate-resistant variety well-suited to the specific conditions of the region. Additionally, the statistical analysis revealed significant correlations between plant density and yield, indicating that the application of fertilizers and biostimulants can contribute to achieving higher yields. Recommendations for yield optimization: optimizing sowing density to 70-72 plants/m² to maximize yield, using fertilizers and biostimulants to enhance plant vigor and production, carefully monitoring climatic conditions and adapting cultivation technologies to drought, introducing complementary varieties for genetic diversification and production stability and implementing sustainable agricultural practices for soil conservation and maintaining fertility.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

QUINOA (*Chenopodium quinoa* Willd.): A PROMISING NEW CROP FOR ROMANIA

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Abstract

This work was undertaken to evaluate the adaptability of quinoa under the South-Eastern Romania's climatic conditions. The trial was carried out in the experimental field of the University of Agronomic Sciences and Veterinary Medicine of Bucharest during the 2024 growing season. For this purpose, two quinoa cultivars sourced from Quinoa Quality ApS, Denmark, were analyzed for growth and seed yield parameters. The experiment was of a monofactorial type and was organized using a Randomized Block Design with four replications. Results showed that in the extremely dry year of 2024, seed yields ranged from 1.000 to 1.917 t/ha. Among the two cultivars, Vikinga yielded the least at 1.000 t/ha, which was 31.44% lower than the control, while the highest yield was recorded for the Puno at 1.917 t/ha, representing a significant increase of 31.43% compared to the control. Both varieties exhibited superior performance across all growth traits, including plant height, maturity, dry weight, and harvest index, all significantly correlated with seed yield. The promising agronomic performance of these cultivars under drought conditions suggests substantial potential for quinoa cultivation in Romania.

Key words*: Chenopodium quinoa, growth traits, seed yield, correlation.*

INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd.) is an Andean plant that originated in the Southern Altiplano of Bolivia and the Puno Region in Peru, near Lake Titicaca, at elevations ranging from 3,000 to 4,000 meters above sea level (Espindola, 1986). Due to its recognized nutritional value and ability to adapt to various geographic regions, quinoa cultivation has expanded globally since the 2000s (Didier et al., 2021). Furthermore, because of its high content of proteins, lipids, vitamins, and minerals, along with an excellent balance of essential amino acids, its consumption has been promoted in many countries facing food security challenges. Quinoa is an annual dicotyledonous plant that is predominantly self-pollinated, although cross-pollination can occur at rates of up to 10 to 15% (Rane et al., 2019). It is a C3 crop recognized for its resilience in tough environments and extreme climatic conditions (Ahmadi et al., 2019; Razzaghi et al., 2011). According to Jacobsen et al. (2003), quinoa demonstrates a high degree of resilience to several major adverse factors, including frost, soil salinity, drought, diseases, and pests. This species is increasingly being regarded as a viable alternative to help address water scarcity issues in various regions around the globe (Pathan et al., 2022; Graziano et al., 2022). As a result, quinoa is recognized as one of the world's most sustainable crops due to its adaptability to diverse environments and its relatively low environmental impact compared to many other crops. Despite its advantages, quinoa remains a neglected and underutilized crop in Romania, highlighting a pressing need for extensive scientific research to facilitate its widespread cultivation. One of the primary challenges in adopting quinoa as a viable agricultural product in our country is the limited availability of suitable genotypes for local production. In South America and several European countries, including the United Kingdom, France, Germany, the Netherlands, and Denmark, various cultivars and ecotypes of quinoa have been successfully developed and cultivated, showcasing a diverse genetic pool that can be harnessed to improve agronomic performance. To enhance quinoa cultivation in Romania, it is essential to identify and introduce suitable genetic materials that are adapted to our specific agro-ecological conditions. Research indicates that quinoa yields can vary

significantly based on genotype and environmental factors (Miranda et al., 2012). Therefore, comprehensive studies aimed at evaluating the performance of different quinoa genotypes across diverse ecological settings in Romania are crucial. These studies should focus on assessing factors such as adaptability, yield potential, disease resistance, and nutritional quality. By conducting localized trials and optimizing genotype selection, Romania can leverage the agronomic potential of quinoa, ultimately promoting crop diversification, improving food security, and enhancing the resilience of our agricultural systems.

Thus, the objective of this study was to assess the adaptability of quinoa (*Chenopodium quinoa* Willd.) to the climatic conditions of South-Eastern Romania by evaluating two Danish quinoa cultivars for their growth and yield-related parameters. The goal was to provide information about crop management under Romanian conditions and to determine which cultivars are worth keeping for further experimentation.

MATERIALS AND METHODS

Plant material and experimental site

This study used two Danish quinoa cultivars, Puno and Vikinga. The main traits of the quinoa cultivars are presented in Table 1. A field experiment was carried out at the University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania during the 2024 growing season. Bucharest is situated in the south eastern corner of the Romanian Plain (44°26' N latitude, 26°06' E longitude and 90 m altitude above sea level).

The soil characteristics of the experimental plots are included in Table 2. The soil was characterized by a slightly alkaline pH, low humus content, moderate levels of total nitrogen, high levels of mobile phosphorus, and very high levels of mobile potassium.

The soil analyses were conducted in 2023 at the laboratories of the National Institute of Research and Development for Pedology, Agrochemistry, and Environmental Protection in Bucharest, Romania.

Table 1. Quinoa cultivars tested, their origins and main traits

Cultivar	Origin	Traits
Puno	Quinoa Quality ApS, Denmark	Drought and salt tolerant; Resistant to downy mildew; High protein quality; High iron content.
Vikinga	Quinoa Quality ApS, Denmark	Drought and salt tolerant; High protein quality; High iron content; Low saponin, integral grain.

Source: https://www.quinoaquality.com/quinoa-seeds

Table 2. Soil characteristics at the study site

Type	Reddish Preluvosol
Texture	Clay-Loam
pH	7.79
Humus content	1.67 %
Total nitrogen	0.166 %
Mobile phosphorus	P_{AL}= 92 mg/kg
Mobile potassium	K_{AL}= 329 mg/kg

Weather conditions

The year 2024 was the warmest year in the history of meteorological measurements in Romania, with an average annual temperature of 12.9916°C, according to the National Meteorological Administration (NMA). Regarding the climatic conditions in Bucharest, the agricultural year 2024 was exceptionally warm with low precipitation throughout the entire growing season (Figure 1). Water stress and heat have had a negative impact on the majority of agricultural crops, leading to a reduction in seed production, both in quantitative and qualitative.

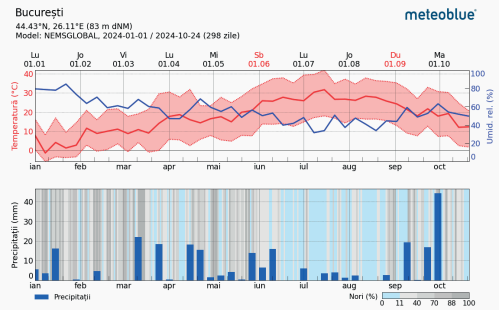


Figure 1. Climate conditions (average temperature and precipitation) in Bucharest - January-October 2024 (Source: https://www.meteoblue.com/ro)

Experimental Protocol and Cultivation Management

In preparation for the soil for sowing spring crops (cultivars of quinoa), plowing was carried out in the fall at a depth of 30 cm. In the spring, to prepare the seedbed (breaking up and leveling the land), on March 11, 2024, work was done with a disc (2 passes) and with a cultivator. The marking of the experimental plot was carried out on March 20, 2024.

The field experiment was of a monofactorial type and was organized using a Randomized Block Design with four replications for each plot/variety (10 m² per plot per replication).

Sowing is a crucial step, as it directly influences seedling emergence, plant density, and ultimately the final yields. Seeds were manually sown on April 9, 2024 and covered to a depth not exceeding 2 cm as recommended by previous research (Hirich et al., 2014).

Plots size was 6 rows of 4 m, with an inter-row spacing of 50 cm, maintaining a density of 100 plants per square meter. However, quinoa is a relatively new crop in Europe, and determining the generally accepted sowing density for its cultivation is challenging (Trotsenko et al., 2023). Different factors, such as local growing conditions, variety of quinoa, and specific farm management practices, may influence the ideal sowing density. Experimentation and observation over time will help refine these practices.

In the spring of 2024, nitrogen (NH4NO3) was applied in split applications, at sowing (40 kg/ha) and during the vegetative growth phase before flowering (40 kg/ha). No chemical treatments were used to control weeds or diseases. During the growing season, three mechanical hoeings were carried out to combat weeds. Additionally, two irrigations were applied, one in April after sowing and the second before flowering

Parameters Estimated

a) Plant height was measured in the field at physiological maturity. For this assessment, ten plants from each replication were randomly selected for evaluation.

b) Maturity was recorded in days, calculated from the date of emergence to the date when the crop was deemed ready for harvest.

c) Dry weight (g/plant) refers to the dry biomass above ground for each individual plant.

The sample size consisted of 5 plants, randomly selected from each replication

d) The harvest index is expressed as the percentage ratio of seed yield per plant to the dry weight per plant.

e) Pathogen and insect control: Visual inspections were conducted to assess the presence of pathogen and insect attacks on quinoa plants.

f) Seed Yield: The seed yield from all the plants in each plot was collected and weighed, with the total yield for each plot converted to tonnes per hectare (t/ha). The harvesting was done manually for each replication when the plants reached full maturity (seed moisture at 12%).

Statistical analysis

The collected data underwent statistical processing, where analysis of variance (ANOVA) was conducted for each parameter at a significance level of 0.05. Additionally, correlation analysis was performed to assess the relationships between seed yield and various growth traits, including maturity, dry weight, plant height and harvest index.

RESULTS AND DISCUSSIONS

Phenological observations

Table 3 presents phenological observations that are critical for assessing the days to maturity.

Table 3. Quinoa cultivars tested and phenological data for 2024 cropping season

Cultivar	Sowing date	Emergence date	Harvest date
Puno	09.04.2024	23.04.2024	03.09.2024
Vikinga	09.04.2024	24.04.2024	02.09.2024

Given the drought conditions during the sowing period, the time from sowing to emergence was 15 days for the Puno cultivar and 16 days for the Vikinga cultivar. Similar findings regarding the period from sowing to emergence for quinoa were reported by Domingos and Bilsborrow in 2022. They found that crop emergence began 10 to 14 days after sowing, with stem elongation and inflorescence emergence occurring at the beginning of June, and flowering taking place at the end of June in all analyzed years.

Resistance to biotic stress

In terms of quinoa cultivars' resistance to biotic stress, both cultivars experienced aphid infestations during their growth and development, although the level of pest pressure was not deemed significant. From the perspective of pathogen attacks, particularly concerning *Peronospora farinosa* f.sp. *chenopodii* (downy mildew), a notably incidence of infection was observed in the Vikinga cultivar, which demonstrated susceptibility to this pathogen. In contrast, the Puno cultivar exhibited a minimal response to the pathogen. The response to downy mildew infection involves visually assessing the severity of the disease, which is determined by the proportion of leaf tissue affected by lesions caused by the pathogen (Danielsen et al., 2004). Zala and Szilagyi (2024) found that the disease severity index recorded an intensity of 6.6% for the Vikinga cultivar and 0.65% for the Puno cultivar.

Plant height and Dry weight

In terms of plant height, there was a significant difference between the two quinoa cultivars. Thus, Puno had the highest measurement at 164.20 cm, while Vikinga measured 114.18 cm (Table 4).

The differences in plant heights among the cultivars may be attributed to their genetic structures and varying responses to the environment. T

he comparison of the dry weight trait between the two varieties is statistically insignificant. Dry weight ranged from 29.03 to 28.07 g/plant, with a mean of 28.55±1.17 (Table 4).

Table 4. Plant height and Dry weight of 2 quinoa cultivars

Cultivars	Plant height (cm)	Dry weight (g/plant)
Puno	164.20	29.03
Vikinga	114.18	28.07
Mean ± S.E*.	139.19±1.78	28.55±1.17
$LSD_{0.05}$**	2.69	3.02

*S.E. - standard error;
**LSD - least significant difference; P ≤ 0.05%.

Maturity and Harvest index

Maturity ranged from 132 days (Vikinga) to 134 days (Puno). The difference between the two cultivars is not significant.

Quinoa is classified as a short-day plant, meaning that it initiates flowering and matures as the length of daylight decreases during the summer months. This photoperiod sensitivity is crucial for its growth cycle.

Results show that the harvest index ranged from 49.27% to 51.33%, with an average of 50.30±3.01, with Puno exhibiting the highest value (51.33%) (Table 5).

Table 5. Maturity and Harvest index of 2 quinoa cultivars

Cultivars	Maturity (days)	Harvest index (%)
Puno	134.00	51.33
Vikinga	132.00	49.27
Mean±S.E.*	133.00±2.13	50.30±3.01
$LSD_{0.05}$**	4.04	5.89

*S.E. - standard error;
**LSD - least significant difference; P ≤ 0.05%.

Seed yield

The mean seed yield values are presented in Table 6. The performance of quinoa varied significantly between the two cultivars under Romanian conditions. Seed yield ranged from 1.000 to 1.917 t/ha, with Vikinga showing the lowest yield at 1.000 t/ha, which was 31.44% lower than that of the control.

The highest seed yield was exhibited by Puno, at 1.917 t/ha, representing a significant increase of 31.43% compared to the control (Mean of experiment).

Table 6. Seed yield of quinoa cultivars

Cultivar	Seed yield (t/ha)	Relative yield (%)	Difference±CT* (t/ha)
Puno	1.917	131.43	+0.4585
Vikinga	1.000	68.56	-0.4585
Mean±S.E**	1.4585±0.256	100	CT
$LSD_{0.05}$***	0.4025	-	-

*CT - Control - Mean of the experiment;
**S.E. - Standard Error;
***LSD - least significant difference; P ≤ 0.05%.

Correlation Analysis

The correlation coefficients are included in Table 7. Seed yield showed a significant positive correlation with all key factors, including plant height, maturity, dry weight, and harvest index. This suggests that taller plants, those that reach maturity later, and those with higher dry weights and harvest index tend to produce greater seed yields. Understanding these relationships can

assist in the selection and breeding of plant varieties that maximize seed production.
The plant height was associated with the duration of maturity, and generally, shorter varieties exhibited earlier traits.
Similar results have also been reported by Tan and Temel (2018).
Regarding the significant positive association between quinoa seed yield and plant height, dry weight, and harvest index, comparable findings have been documented by Bhargava et al. (2007) and Szilagyi et al. (2014).

Table 7. Correlation coefficients among five traits in quinoa cultivars

Traits	Maturity	Plant height	Dry weight	Harvest index	Seed yield
Maturity	x				
Plant height	0.425**	x			
Dry weight	0.143	0.321*	x		
Harvest index	0.081	0.218*	0.418**	x	
Seed yield	0.462**	0.429**	0.633**	0.518**	x

Level of significance: *P = 0.05, **P = 0.01

CONCLUSIONS

Based on the data collected during the first year of testing the Puno and Vikinga cultivars, we can conclude that quinoa has the potential to be a viable alternative crop with favorable characteristics for cultivation in the pedo-climatic conditions of Romania. However, it is essential to test earlier sowing dates (specifically, the end of March) in the coming year. By selecting the optimal sowing time, the plants are likely to develop more robustly, leading to increased seed production capacity and an earlier harvest (starting in early August). Additionally, it is important to identify the most effective treatments for combating downy mildew.

ACKNOWLEDGMENTS

This work was supported by project ADER 115/18.07.2023, funded by the Ministry of Agriculture and Rural Development, Romania. We would also like to express our gratitude to Quinoa Quality ApS, Denmark, for providing the seeds of Puno and Vikinga cultivars.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

IMPROVING SOYBEAN QUALITY PARAMETERS BY SOWING ON DIFFERENT DATES IN VARIOUS CLIMATIC YEARS

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Abstract

Given the challenges posed by current climate change, which impacts food supply chain, alongside the growing global population, rising food demand, and continuous environmental degradation, it is essential to identify crop varieties that are stable in both productivity and quality. A field experiment was carried out at the Research and Development Station for Agriculture (RDSA) Turda over a period of four years (2021-2024), each representing different climatic conditions. The study included six soybean varieties of different maturity groups developed at RDSA Turda: Felix, Iris TD, Ziana TD, Raluca TD, Isa TD, Miruna TD. The soybean varieties were sown on two different soil temperatures (5°C and 7°C), based on readings taken at 8 a.m. The quality response of the soybean varieties sown at different soil temperatures was influenced by both genetic factors and the sowing date. These findings emphasize the potential of adjusting sowing dates to improve soybean quality, providing valuable guidance for farmers aiming to optimize performance in similar agroecological conditions.

Key words*: climatic conditions, sowing date, soil temperature, soybean, quality.*

INTRODUCTION

Soybean (*Glycine max* [L.] Merr.) is one of the most important food, forage, and agro-technical crops (Negrea et al., 2023), but it is highly sensitive to environmental factors and the applied technology. The development of soybeans is influenced by various environmental factors, such as temperature, precipitation, relative humidity, soil moisture, and especially photoperiod (Ávila et al., 2003).

One of the most important factors determining soybean crop yield, which can be implemented in current technology without additional costs, is the sowing date. The sowing date is a critical factor affecting the yield and quality of soybeans (Rahman et al., 2005), with early sowing being considered significant in the scientific literature for plant weight, leaf area index, and growth rate (Öztürk and Sögüt, 2018). Currently, the optimal sowing date for soybeans in Europe is within a broad range, from mid-April to mid-May (Dima, 2016), and according to some authors, soybean sowing should be done when the daily average soil temperature reaches 8ºC (Jarecki and Bobrecka-Jamro, 2021).

The soybean crop is currently considered the richest and cheapest source of proteins, minerals, vitamins, and high-quality fats (Sheshama et al., 2016). The composition of soybean seeds is an important factor in determining the economic value of soybean beans for commercialization (Brumm and Hurburgh, 2006). The global production of oil and protein is based on soybean seeds, due to their high protein and fat content, averaging 40% and approximately 20%, respectively (Stein et al., 2008).

In recent years, as consumers become increasingly aware of the health benefits of soybeans, there is growing interest in breeding programs aimed at improving taste and health benefits (Muntean et al., 2019).

In the context of climate change and the growing demand for food, this study aims to provide answers regarding the influence of changes in sowing date and soybean varieties from different maturity groups on the chemical composition of the seeds.

MATERIALS AND METHODS

In order to assess the quality of soybean seeds, an experiment was set up at the Agricultural Research and Development Station (ARDS) Turda over four different agricultural years

(2021-2024), aiming to study the influence of the sowing time on the main quality parameters. The study was carried out on a Vertic Phaeozem soil, typical of the Transylvanian Plateau. Chemically, the soil is weakly alkaline with a neutral pH, contains a moderate to high level of humus, is well supplied with nitrogen and potassium, and has a medium phosphorus content.

The biological material studied consisted of six soybean varieties developed and registered at Turda. With the exception of the early soybean variety Felix, which was registered in 2005, the remaining five varieties were registered after 2017, making them the most recent soybean varieties developed at Turda. Among them, the Isa TD variety is classified as very early, while the Iris TD, Ziana TD, and Miruna TD varieties are considered early-maturing genotypes. The Raluca TD variety, registered in 2019, is a semi-early soybean variety and is the latest genotype included in the experiment. Primary soil preparation consisted of plowing, which was performed in the autumn following the harvest of the preceding crop. The seedbed was then prepared two days prior to sowing. The crop was sown at a rate of 550,000 seeds per hectare, with a row spacing of 0.5 meters. Weed management was carried out through two mechanical hoeing operations conducted during the vegetation period. In the field, the experiment was conducted using the split-plot design with three replications, with the sowing time selected based on the soil temperature measured at 8:00 AM. Two different temperatures were chosen: 5°C (early sowing) and 7°C (optimal), with each temperature being measured over three consecutive days before sowing. In the laboratory, using near-infrared spectrophotometry, the seeds from the experimental variants were analyzed for protein content, oil content, and four fatty acids: stearic acid, oleic acid, linoleic acid and linolenic acid. Data processing was performed using the Past4 and Microsoft Excel programs.

Over the past four years, Turda has experienced notable variations in temperature, with a clear trend toward warmer weather, especially during spring and summer months (Table 1).

In 2021, spring was notably cooler than average, especially in April, which was 2.1°C below the multiyear norm, but the summer brought a brief heat surge, with July rising 3°C above average. The year 2022 followed with a mild spring and a significantly warmer summer, marked by a July deviation of +3.3°C and an exceptionally warm October (+2.6°C). In 2023, temperatures were generally above average, with an unusually hot autumn, September and October recorded deviations of +3.8°C and +4.2°C, respectively, indicating a prolonged summer season.

The year 2024 was the warmest of all, with every month from March to October exceeding the multiyear average; March alone was 4.4°C warmer than usual, and July reached +4.2°C, with negative impact on soybean crop development, soybean yield and quality.

In terms of rainfall registered in Turda (Table 2), a significant interannual variability was identified, with periods of both drought and intense precipitation. In 2021, rainfall was close to average overall, with high values in May and July, but June and October were markedly dry, showing deficits of -39.8 mm and -24.0 mm, respectively.

The year 2022 was highly irregular: while spring and early summer were generally drier, especially July, with 52.8 mm less than average, late summer and early autumn brought heavy rainfall, particularly in August and September, which exceeded norms by +38.5 mm and +77.5 mm.

In contrast, 2023 saw an extremely wet June (+59.9 mm), along with consistently high rainfall in late summer and early autumn. However, the spring months were notably dry, with May recording 36.2 mm less than average. The year 2024 returned to predominantly dry conditions, with June experiencing a major shortfall of -48.4 mm and several months showing rainfall below average, although September stood out slightly wetter (+21.8 mm). Overall, the four-year period reflects increasing variability in rainfall distribution, highlighting irregular seasonal patterns and the growing unpredictability of precipitation, with negative impact on soybean yield and quality.

Table 1. Monthly average temperatures and deviations from multiyear average in Turda (2021-2024)

Year	Temperature (°C)	Month							
		III	IV	V	VI	VII	VIII	IX	X
2021	Average	3.3	7.8	14.1	19.8	22.7	19.7	15.0	9.7
	Deviation from multianual average	-1.4	-2.1	-0.9	1.9	3	0.4	-0.1	0.2
2022	Average	3.6	8.8	16.3	21.1	23.1	22.3	14.3	12.4
	Deviation from multianual average	-0.8	-1.2	+1.3	+3.1	+3.3	+2.8	-0.9	+2.6
2023	Average	6.3	8.8	15.4	19.0	21.8	22.1	19.0	14.0
	Deviation from multianual average	1.9	-1.2	0.4	1.0	2.0	2.6	3.8	4.2
2024	Average	8.8	13.3	15.8	21.7	24.0	23.4	17.9	11.7
	Deviation from multianual average	4.4	3.3	0.8	3.7	4.2	3.9	2.7	1.9

Table 2. Monthly rainfall and deviations from multiyear average in Turda (2021-2024)

Year	Rainfall (mm)	Month							
		III	IV	V	VI	VII	VIII	IX	X
2021	Sum	27.3	38.4	80.8	45.0	123.1	52.9	39.1	11.6
	Deviation from multianual average	3.7	-7.5	12.1	-39.8	46	-3.6	-3.4	-24.0
2022	Sum	8.3	42.5	82.9	41.8	25.2	94.6	119.9	16.3
	Deviation from multianual average	-16.0	-3.1	+13.5	-42.8	-52.8	+38.5	+77.5	-19.1
2023	Sum	10.8	30.5	33.2	144.5	85.8	98.5	116.1	19.8
	Deviation from multianual average	-13.5	-15.1	-36.2	59.9	7.8	42.4	73.7	-15.6
2024	Sum	37.7	38.8	60.7	36.2	49.3	36.7	64.2	14.4
	Deviation from multianual average	13.4	-6.8	-8.7	-48.4	-28.7	-19.4	21.8	-21.0

RESULTS AND DISCUSSIONS

The chemical composition of soybeans is influenced by a range of factors. These factors can include environmental conditions, soil quality, cultivation practices, and the specific variety of soybeans, all of which impact the overall nutrient profile and quality of the seeds. One of the most crucial aspects determining the crop's end use is the content of proteins and fats in the seeds.

These components are particularly important when it comes to utilizing soybeans in various food and animal feed industries. Furthermore, fatty acids, offer significant nutritional benefits for human health. Soybeans are especially valued for their ideal balance of unsaturated, monounsaturated, and polyunsaturated fatty acids, making them a beneficial source of fats for the human body.

Figure 1 shows the variation in oil content, averaged over the four experimental years. This analysis covered five soybean varieties from three distinct maturity groups (very early-000, early-00, semiearly-0).

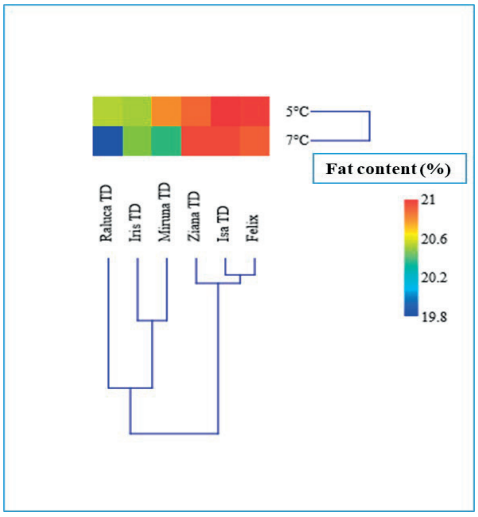


Figure 1. Fat content obtained in five soybean varieties depending on soil sowing temperature (Turda, 2021-2024)

The variation in oil content was observed in relation to the soil temperature at sowing time, as represented by the heat map. Warm colours correspond to higher values, while cool colours indicate a lower fat concentration in the seeds. The oil content varied between 19.8% and 21%, with the minimum of the experiment observed in the latest maturing soybean genotype (Raluca TD), when sowing was done at the optimal temperature (7°C).

Generally, higher fat content values were identified when soybeans were planted at 5°C, with the highest value of 21% reached by the very early-maturing Isa TD variety. Based on cluster analysis, it can be seen that the varieties Ziana TD, Isa TD, and Felix exhibited similar behaviour, showing high fat content values for both sowing dates. For the Miruna TD variety, an increase higher than 0.5% in fat content was observed when sowing was done early, compared to the optimal temperature, similar results were also obtained by Assefa et al. (2019), who found that delayed planting led to a decrease in oil content.

A greater variation in protein content was noted across the different soybean genotypes, with the genetic factor playing a more significant role than the sowing time (Figure 2).

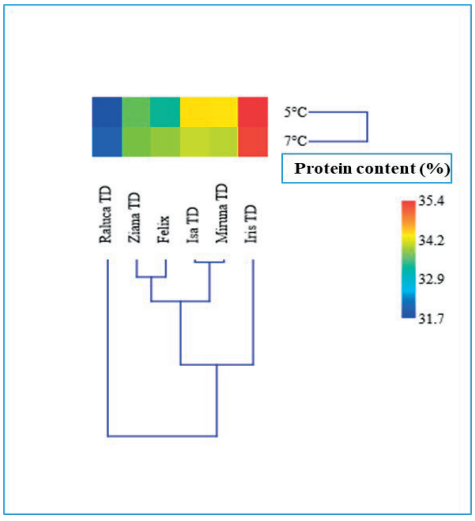


Figure 2. Protein content obtained in five soybean varieties depending on soil sowing temperature (Turda, 2021-2024)

The semi-early variety Raluca TD exhibited protein values below 32% in both experimental variants, while the highest protein content of 35.4% was found in the early-maturing variety Iris TD. Regardless of the sowing temperature, cluster analysis revealed similar patterns for the varieties Ziana TD and Felix TD, as well as for Isa TD and Miruna TD. The Raluca TD and Iris TD varieties did not form clusters and remained independent, as they represented the extremes of the experiment in terms of protein content. The research results conducted by Sadeghi and Niyaki (2013) showed that earlier sowing of soybeans improved seed quality compared to delayed sowing. As planting was delayed, the protein content tended to decrease in early-maturing varieties, but remained stable between sowing dates in varieties from other maturity groups (Morris et al., 2021).

The analysis of the four fatty acids indicates a considerable variability, both depending on the variety analysed and the sowing date (Figure 3). Varga et al (2024), following their studies, concluded that the fatty acid profile varied depending on the sowing date, with higher results for oleic acid and linolenic acid obtained from delayed sowing of the crop.

An improvement in stearic acid content (+0.21%) was observed in the very early soybean variety Isa TD when sown early compared to the optimal sowing date. For the Felix variety, sowing at the optimal time led to a significant increase in oleic acid content, which reached 26% in this experimental variant. As for linoleic acid, with the exception of the Raluca TD variety, where maximum values of the experiment were reached (>55%) at both sowing dates, the other varieties studied showed a similar profile, with much lower values compared to the latest variety in the experiment. The content of linolenic acid varied the most within the experiment. It ranged from 3.58% to 7.2%, with higher values generally identified when soybeans were sown at 7°C. The maximum value of the experiment was observed in the early soybean variety Miruna TD, sown at the optimal time, while Raluca TD exhibited the lowest values of the experiment at both sowing dates.

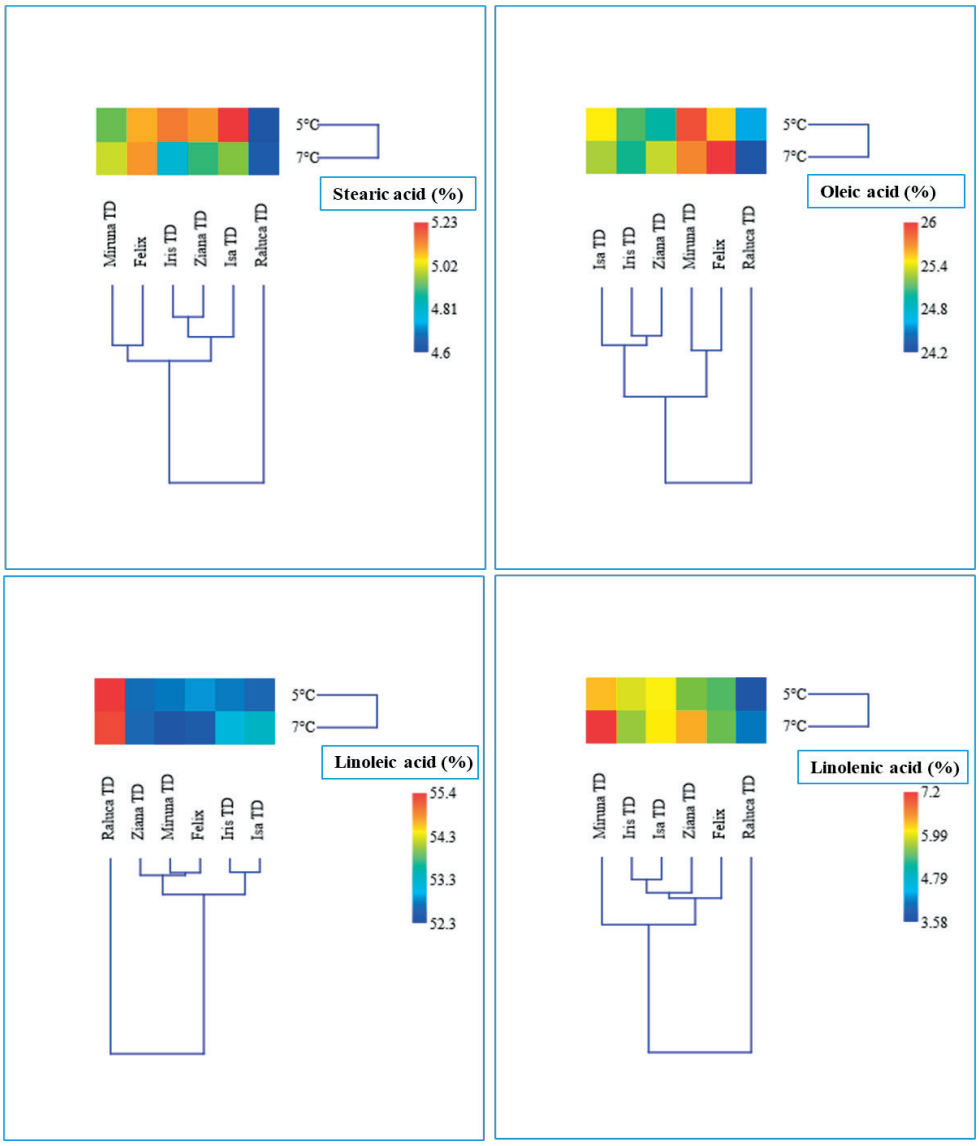


Figure 3. Fatty acid profile obtained in five soybean varieties depending on soil sowing temperature (Turda, 2021-2024)

Although seed quality is genetically determined, it exhibits considerable variability depending on environmental conditions and agronomic practices. The development of a new soybean cultivar is a time-intensive process, the identification of technological factors that could enhance seed quality being essential for sustainable agriculture. Using the method proposed by Eberhart and Russell (1966) the stability of soybean seed quality can be assessed in relation to the climatic conditions over the four experimental years and varying sowing dates.

Figure 4 illustrates the stability of oil content in the six soybean cultivars studied. When soybean was sown at a soil temperature of 5°C, it was evident that in 2022, the seeds had lower oil content compared to the other three experimental years. Notably, the very early-maturing cultivar Isa TD demonstrated superior performance relative to the other four cultivars under unborable environmental conditions.

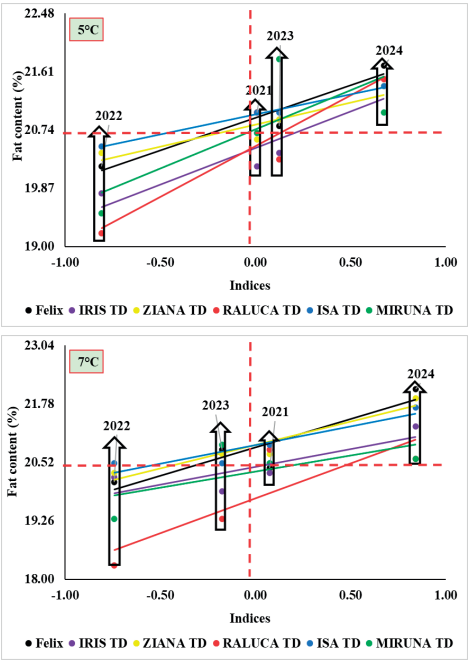


Figure 4. Fat content stability based on environment indices (Turda, 2021-2024)

When soybeans were sown at a soil temperature of 7°C, the varieties experienced optimal conditions for oil accumulation in the seeds only in the years 2021 and 2024, with lower oil content observed in the other two years.

The strongly positive trend of the regression lines indicates that oil content in soybean seeds showed significant variability across the four experimental years. When soybean was sown at the optimal temperature, a shallower trend of the regression lines was observed, with the exception of the Raluca TD cultivar. The remaining four cultivars exhibited more consistent oil content. Despite the improved stability of this quality parameter when soybeans is sown at the optimal time, seeds sown early generally contained higher oil levels. It is important to highlight that, irrespective of the sowing date, the year 2024 proved to be the most favourable for oil yield in the soybean crop.

Based on the stability analysis of protein content in the six soybean cultivars grown across four distinct growing seasons and at two different sowing dates (Figure 5), a more pronounced stability was observed in the Raluca TD cultivar when was sown at a soil temperature of 7°C, as well as in the early-sown Iris TD cultivar when was sown at a soil temperature of 5°C. In this case, the regression lines were nearly parallel to the x-axis. A gentler trend was observed for the other cultivars at both sowing dates, indicating a good level of stability in protein content, though with relatively lower values. However, protein content exhibited considerable variation primarily due to the genetic factor. Across all experimental years and sowing dates, the early-maturing Iris TD cultivar consistently demonstrated superior performance. This cultivar appears to be the most advantageous in terms of seed protein content. Nevertheless, over the four experimental years, protein content remained relatively low, with values consistently below 40%.

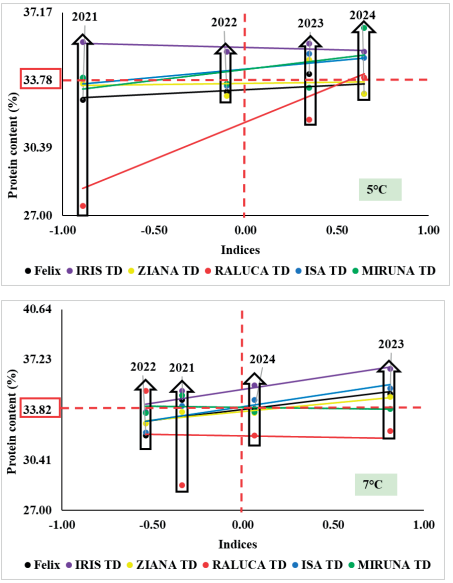


Figure 5. Protein content stability based on environment indices (Turda, 2021-2024)

Oil accumulation in soybean seeds occurs during the grain filling stage, with the highest levels typically reached around 30 days post-flowering (Wilson, 2004). Regardless of the cultivar studied or the experimental year, the beginning of flowering date was noted between June 15 and July 10. Therefore, the drought occurring in July and August, along with the high temperatures, could have affected the oil content in soybean seeds. Although for 2024 the total precipitation in July and August was lower compared to the other three years, its

distribution throughout the reproductive stage, along with lower temperatures, led to the highest fat content observed in the soybean seeds. The year 2022 was unfavourable for lipid accumulation in the seeds. Although the total precipitation reached 120 mm over the two months, it was mainly concentrated in the first decade of July and the first and last decades of August. Additionally, the average temperature during these months exceeded the long-term mean by more than 3°C, leading to small values for fat content (Figure 6).

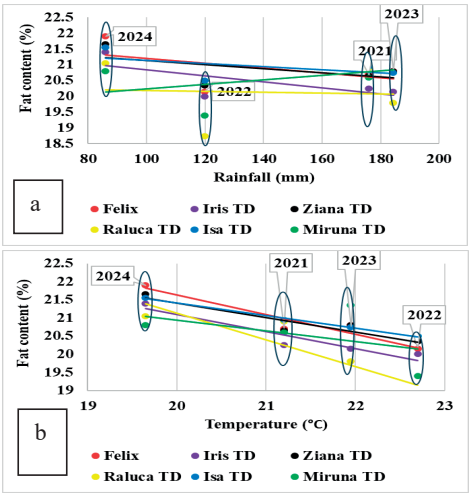


Figure 6. The relationship between oil content and the total precipitation in July and August (a), as well as between oil content and the average temperature in July and August (b) (Turda, 2021-2024)

In terms of protein content analysis in relation to total precipitation during the last two summer months and the average temperature during that period, it is evident that this quality parameter was more significantly influenced by the genetic factor. The Raluca TD variety was the only genotype that exhibit greater fluctuations in protein content, likely due to its notably longer growing season compared to the other four cultivars studied (Figure 7). The data obtained by Księżak and Bojarszczuk (2022) showed that delaying sowing until the first week of May, along with limited precipitation during the growing season, leads to the accumulation of proteins in the seeds. Other authors, such as Sheshama et al. (2016), obtained different results, namely a decrease in protein content with delayed sowing, which could be attributed to the reduced duration of the reproductive phase due to fewer days until maturity, and this may have also affected the synthesis of seed fats.

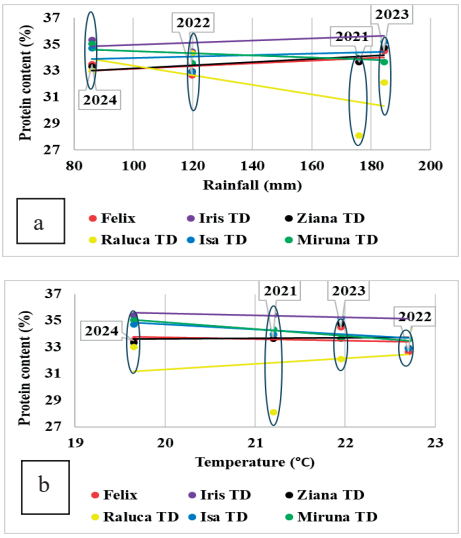


Figure 7. The relationship between protein content and the total precipitation in July and August (a), as well as between protein content and the average temperature in July and August (b) (Turda, 2021-2024)

Robinson et al. (2009) emphasized that earlier sowing of soybeans resulted in an increase in protein content, while a decrease in fat content was observed in the harvested seeds.

CONCLUSIONS

The study highlights that oil content in soybeans is significantly influenced by sowing temperature, with lower soil temperatures (5°C) leading to higher fat concentrations, particularly in early-maturing varieties like Isa TD. Additionally, the Raluca TD variety, which has a longer growing season, exhibited the lowest oil content when sown at the optimal temperature (7°C). Cluster analysis further confirmed that certain varieties, such as Ziana TD, Isa TD, and Felix TD, consistently exhibited high oil content across both sowing dates. These findings highlight the significance of selecting optimal sowing temperatures and varieties to enhance oil content, a key factor in optimizing soybean oil yield.

Protein content in soybean seeds is primarily influenced by genetic factors, with significant

variation observed across different genotypes. The semi-early Raluca TD variety consistently showed lower protein content, while the early-maturing Iris TD variety exhibited the highest values. Cluster analysis revealed that certain varieties shared similar protein content patterns, while Raluca TD and Iris TD stood out as extremes, demonstrating the critical role of genetic traits in determining protein accumulation in soybeans.

Fatty acid content in these six soybean cultivars varied significantly based on both variety and sowing date. Early sowing improved stearic acid in Isa TD and oleic acid in Felix. Raluca TD consistently had the highest linoleic acid, while linolenic acid showed the most variation, with higher levels at 7°C sowing. These findings highlight the influence of both genetic and environmental factors on soybean fatty acid composition.

In term of stability, soybean seed quality, influenced by both genetic and environmental factors, showed significant variability across the four experimental years and different sowing dates. The Isa TD cultivar performed well under challenging conditions, while the Raluca TD and Iris TD varieties exhibited stable oil and protein content, respectively. Optimal conditions for oil accumulation were observed when sowing occurred at 7°C in 2021 and 2024, with the year 2024 being the most favourable. Despite higher stability in protein and oil content at the optimal sowing time, earlier sowing generally resulted in higher oil content, highlighting the importance of environmental and agronomic practices in enhancing seed quality.

The experimental data revealed that oil accumulation in soybean seeds is primarily influenced by environmental conditions during the grain filling stage. While precipitation and temperature variations in July and August impacted oil content, the distribution of precipitation and cooler temperatures in 2024 resulted in the highest fat content. Conversely, 2022, with higher temperatures and uneven rainfall, was unfavourable for lipid accumulation. Regarding protein content, genetic factors played a more significant role, with the Raluca TD variety exhibiting greater fluctuations due to its longer growing season.

ACKNOWLEDGEMENTS

This work was financed by a grant from the Ministry of Agriculture and Rural Development through the ADER 2023-2026 program, project no. 1.4.1/19.07.2023 - Improving the methods of weed control in soybean culture, to increase economic efficiency by reducing the number of treatments and the negative impact on the environment

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

STUDY OF UNSTABLE PHENOTYPIC BEAN SEEDS

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Abstract

Since 2010, at the Vegetable Research and Development Station (V.R.D.S.) Buzău there was initiated a program in order to preserve and valorize local bean population (Phaseolus vulgaris L.) from Eastern and South – Eastern Romania. In 2020, the germplasm collection contained over 450 accessions. This paper presents the study of 3 climbing bean accessions seeds sown. For each accession sown, there were taken into study 9 new biotypes harvested. The seeds sown represented the control variant for the analysis of the harvested biotypes. The seeds were evaluated in what it concerns quantitative and qualitative characteristics. The main colors of the control seeds were brown (V_1 - medium, V_2 - light and V_3 - dark). The seeds harvested presented a lot of colors including white (on entire graine - 1.3.; 1.4.; 2.6.; 3.1 or as secondary color 1.5.; 1.7.; 1.8.; 2.7.; 3.3.). The mean weight of 100 seeds varied between 36.52 g (1.4.) and 70.95 g (1 Mt). The results obtained, corroborated by diversity of color and shape of the seeds harvested, show the presence of cross-pollination of climbing bean plants and increase the value of the bean germplasm collection.

Key words: *cross-pollination, germplasm collection, new biotypes, Phaseolus vulgaris L.*

INTRODUCTION

In Eastern and South-East Romania there are favorable conditions for growing common bean production, as well as a great tradition in cultivation (Ruşti and Munteanu, 2008). Collecting and conserving biodiversity, in what it concerns bean, represents one of the main activity objectives for the Vegetable Research and Development Station (V.R.D.S.) Buzău.

Thus, in 2010 started the collecting of local bean population from the main vegetable areas in Romania. Bean germplasm collection of V.R.D.S. Buzău contains over 450 accessions.

Similar researches were made in Romania by Rădulescu (1940), Munteanu (1985), Stan et al. (1993 and 1995), Leonte et al. (2004), Riviș and Nedelea (2008), Giurcă and Murariu (2009), Danci et al. (2010) and Madoșa et al. (2010 and 2011).

Normally, in Romania, the bean (*Phaseolus vulgaris* L.) is considered a self-pollinated plant. Ali et al. (2020) also has the same opinion. Olaru (1982) and Ciofu et al. (2003) agree with Ali et al. (2020), but they do not exclude cross-pollination. In a normal crop Bliss (1980) and Ruști and Munteanu (2008) estimate that bean is highly self-pollinated, and only 0.2-0.5% cross-pollinated. In Puerto Rico, Vakili (1976), quoted by Bliss (1980), reports that carpenter bees (*Xylocopa brasilianorum* L.) were responsible for levels as high as 15 to 20% cross-pollination. In what it concerns some accessions, over time, the phenotypic instability phenomenon has been more intensive because of some particularities concerning flower (Figure 1) morphology (Debouck and Hidalgo, 1986).

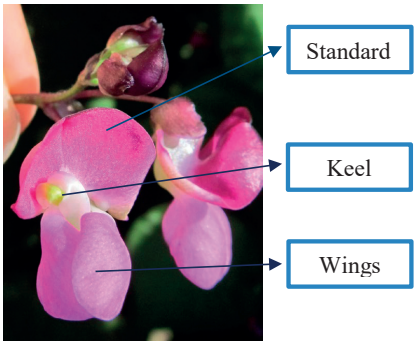


Figure 1. Bean flower morphology (about Teodorescu, 2016)

Studying flower aspect at accessions that presented a high variability percent there was observed that stigma and anthers were no longer protected by the keel. Under the pressure of different insects (especially bees) the stigma get

out of the keel and have contact with the full of pollen bees feet from another flower or it comes off and leaves free the stigma and the anthers (Figure 2). The style turning over inside the keel is foreclosed by the brush of hairs near the stigma and also because of the style scrolling degree.

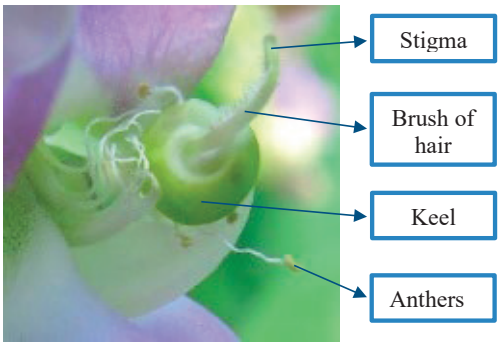


Figure 2. Stigma and anthers were no longer protected by the keel (about Teodorescu, 2016)

At the proveniences that present such flower morphology, the pollination is allogamy and can be made by insects - entomophily and by wind - anemophily (Teodorescu, 2016).
The aim of this paper was to present the effects of cross-pollination within a bean germplasm collection in climatic conditions from Eastern and South-Eastern part of Romania.

MATERIALS AND METHODS

The study was carried out in V. R. D. S. Buzău, Romania (45°9′N and 26°49′E) in 2020.
The biological material (climbing accessions) was cultivated according to the common production technology recommended for this area by the specialty literature (Munteanu et al., 1989; Ciofu et al., 2003; Ruşti and Munteanu, 2008). Therefore, the experimental variants were the following:

V_1		V_2		V_3	
1 Mt	35 B	2 Mt	47 BBD	3 Mt	48 B
1.1.	35 BB	2.1.	47 BBDA	3.1.	48 BA
1.2.	35 BD	2.2.	47 BBDC	3.2.	48 BB
1.3.	35 BE	2.3.	47 BBDD	3.3.	48 BC
1.4.	35 BF	2.4.	47 BBDF	3.4.	48 BE
1.5.	35 BG	2.5.	47 BBDG	3.5.	48 BF
1.6.	35 BH	2.6.	47 BBDH	3.6.	48 BG
1.7.	35 BI	2.7.	47 BBDI	3.7.	48 BI
1.8.	35 BJ	2.8.	47 BBDJ	3.8.	48 BJ
1.9.	35 BK	2.9.	47 BBDK	3.9.	48 BK

This germplasm collection was cultivated in green - house covered with polyethylene. The green house's ventilation space permitted the access of insects.
The determination of plants, pods and seeds characteristics were made according to U.P.O.V. guideline (2015), C.P.V.O. protocol (2009) and color scale (Genchev and Kiryakov, 2005). For characterization, there were used 10 seeds from each accession, according to *Handbook on evaluation of Phaseolus germplasm* (De la Cuadra et al., 2001).
Length determination was made in mm measured in parallel with the hilum, width was measured from the hilum to the opposite side and thickness was measured perpendicular on width in cross section (Figure 3).

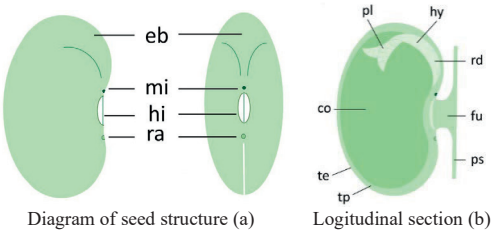


Diagram of seed structure (a) Logitudinal section (b)

Figure 3. Seed Structure of a Dicotyledon **(a)**: eb - embryo bulge; mi - micropyle; hi - hilum; ra - raphe and **(b)**: pl - plumule; hy - hypocotyl; rd - radicule; co - cotyledon; fu - funicle; ps - pod; te - testa; tp - testa perenchyma (about Chen et al., 2021)

A more detailed overview of the plumule – radicle complex includes epicotyl and primary leaves (Debouck and Hidalgo, 1986). Rădulescu (1940) quoted by Olaru (1982) pays particular attention to the coloring area around hilum, otherwise the hilum is always white, the edge surrounding the hilum may have a different coloration with various intensity (absent, weak, medium and strong) to the rest of testa and also it is interrupted at the micropyle and caruncle.
Caruncle (raphe) represents a heart - shaped lift having the tip facing the end of the seed. The stripe of the caruncle represents a narrow band which leaves from the top of caruncle, it extends to the end of the seed - were it ends like a swallow`s tail, and it has a variable length (Figure 4).
According to Descriptor for *Phaseolus vulgaris* (IBPGR, 1982), van Schoonhoven A. and Pastor-Corrales (1994) and Debouck D. (2009) 100 seeds mass were randomly chosen for

measuring seed mass (small - less than 25 g, medium - 25 g to 40 g and large - more than 40 g).

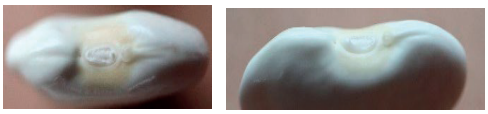


Figure 4. Aspects of the embryo bulge, micropyle, hilum, caruncle and the stripe of the caruncle (about Teodorescu, 2024)

In order to determine seeds weight there was used an electronic balance reading to 0.0001 g (Partner WAS220/x).

The experimental design was a randomized block with 3 replicates for each variant. The seeds sown represented the control variant for the analysis of the harvested biotypes. In order to analyze the results analysis of variance and multiple comparison method Duncan's test were used (Harter, 1960; Săulescu and Săulescu, 1966).

There was used coefficient of variation (CV) because it allows the direct comparison of data sets variation (Ireland, 2010). The coefficient of variation in a single sample with observations is defined as CV = *s/m,* where *m* is mean and *s* is standard deviation (Forkman, 2009). Variability appreciation according to the CV values (Munteanu and Fălticeanu, 2008; Giurcă and Murariu, 2009) was made this way: low variability (CV < 10%), mean (CV = 10- 20%) and high (CV > 20%).

RESULTS AND DISCUSSIONS

In 2020, most of the accessions cultivated presented a high degree of instability.

This paper presents quantitative (100 seed weight, length, thickness and width) and qualitative (shape, seed color, veining and hilum ring - intensity and color) characteristics of seeds harvested to 9 biotypes in comparation with seeds sown (Figure 5).



Figure 5. Brown - main color of the control seeds

The main colors of the control seeds were brown (V_1 - medium, V_2 - light reddish and V_3 - dark). The secondary color was also present at V_1 (black - in streaks), V_2 (reddish brown - in streaks and in patches) and V_3 (reddish brown - in patches).

In what it concerns V_1, (Table 1) it presents maximum values at 1Mt (weight - 70.96 g and thickness - 7.31 mm), 1.1. (length - 17.01 mm), and 1.8. (width - 9.85 mm) and all minimum values at 1.4. (weight - 37.05 g; length - 12.51 mm; width - 6.82 mm and thickness - 5.68 mm CV had a value of 16.15% (bigger than 10% - mean variability) only regarding weight.

Table 1. Main quantitative characteristics of the seeds V_1

V_1	100 seeds mass (g)	Seeds dimensions (mm)			Length/ Width	Width/ Thickness
		Length	Width	Thickness		
1 Mt	70.96 a*	16.04 b	9.23 b	7.31 a	1.74	1.26
1.1.	67.49 a	17.01 a	9.05 b	6.62 bcd	1.88	1.37
1.2.	58.82 bc	13.98 ef	8.88 bc	6.94 abc	1.57	1.28
1.3.	64.48 ab	15.68 bc	8.84 bc	6.68 bc	1.77	1.32
1.4.	37.05 d	12.51 g	6.82 e	5.68 e	1.83	1.20
1.5.	52.73 c	14.16 ef	8.27 d	6.93 abc	1.71	1.19
1.6.	56.39 c	15.07 cd	9.28 b	6.18 d	1.62	1.50
1.7.	64.73 ab	14.73 de	8.93 bc	7.10 ab	1.65	1.26
1.8.	58.82 bc	15.06 cd	9.85 a	6.49 cd	1.53	1.52
1.9.	56.55 c	13.82 f	8.52 cd	6.99 abc	1.62	1.22
Mean	**58.80**	**14.81**	**8.77**	**6.69**	**1.69**	**1.31**
Stand. var.	9.50	1.28	0.81	0.48	0.11	0.12
CV%	16.15	8.61	9.22	7.21	6.71	8.92
Max.	70.96	17.01	9.85	7.31	1.88	1.52
Min.	37.05	12.51	6.82	5.68	1.53	1.19
	LSD 5% = 6.5887 LSD 1% = 9.0360 LSD 0.1% = 12.2989	LSD 5% = 0.7522 LSD 1% = 1.0316 LSD 0.1% = 1.4042	LSD 5% = 0.4281 LSD 1% = 0.5871 LSD 0.1% = 0.7991	LSD 5% = 0.4617 LSD 1% = 0.6332 LSD 0.1% = 0.8619		

*Different letters between variants denote significant differences (Duncan's test, $p<0.05$).

The CV in what it concerns all quantitative characteristics had a value between 6.71% (length/width) and 9.22 %(width); this shows a low variability for the other parameters analyzed.

The appearance of a large number of biotypes represents the result of the presence of numerous insects in the crop (Figure 6).

According to U.P.O.V. guideline (2015), C.P.V.O. protocol (2009) and also to the color scale (Genchev and Kiryakov, 2005), at accessions with kidney – shaped seeds shape, it is recommended to evaluate also degree of curvature (weak, medium or strong)

Same shape with 1 Mt (kidney) it also found at 1.1., 1.3. and 1.8. The predominant main color at V_1 (Table 2) was brown, except 1.3., 1.4. (white) and 1.9. black.

The predominant secondar color (Figure 7) to this variant was black (1Mt, 1.2., 1.6. and 1.7.)

The shape of the secondary striped color was the most frequently met (1Mt, 1.3., 1.6., 1.7. and 1.8.).



Figure 6. Insects present in the crop and flowers after their visit (Teodorescu, 2024).

Table 2. Main qualitative characteristics of the seeds V_1

V_1	Seeds shape[1]	Color			Hilum ring	
		Main	Secondar	Shape of secondar	Intensity	Color
1 Mt	kidney[2] w	brown	black	in streaks	absent	–
1.1.	kidney s	L brown	–	–	weak	brown
1.2.	elliptic	beige	black	in streaks	medium	reddish brown
1.3.	kidney s	white	–	–	absent	–
1.4.	elliptic	white	–	–	absent	–
1.5.	elliptic	L brown	white	embryo bulge – in patches	strong	brown
1.6.	elliptic	brown	black	in streaks	medium	reddish brown
1.7.	elliptic	reddish brown	black white	in streaks in patches	weak	D reddish brown
1.8.	kidney w	L brown	brown white	in streaks in patches	weak	brown
1.9.	elliptic	black	L brown	in fine in patches	absent	–

[1]Shape of median longitudinal section; L=light; D=dark.
[2]Degree of curvature for kidney - shaped only: weak (w), medium (m) and strong (s).

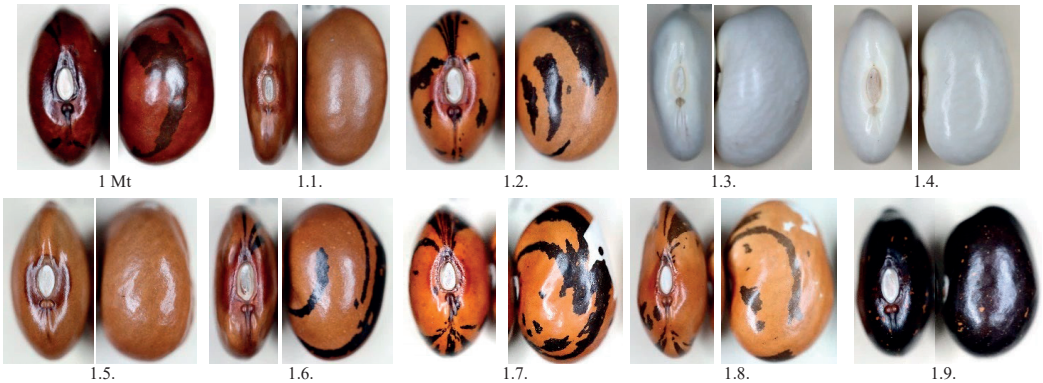


Figure 7. Aspects of qualitative characteristics of the seeds V_1

In what it concerns V_1, the differences in color of the hypocotyl were evident from the first development stages of plants. (Figure 8 a and b). 1.5. draws attention because the secondary color is localized in the embryo bulge area and also because the color hilar ring was strong (Figure 8 c and d). The difference between 1.7. and 1.8. is given by the secondary color in streaks (Figure 8 e). The funicle color varied within this variant from white (Figure 8 f) to brown (Figure 8 g). In the field, sometimes (harveast), the brightness was high.



a b c d e f g

Figure 8. Aspects of crop and seeds belong to V_1: a and b - differences in anthocyanin coloration of the hypocotyl; c - white secondary color on embryo bulge area at 1.5.; d - strong coloration of hilar ring of 1.5.; e - comparation between the secondar color in streaks to 1.7. and 1.8.; f - white funicle at 1.3. and g - brown funicle at 1.1.

At V_2 (Table 3), the two highest values were registered at the control variant (weight - 54.67g and width - 8.98 mm) and two minimum values at 2.8 (weight - 38.61 g; length - 11.41 mm). The mean weight value was 45.50 g and more than half of the new biotypes were below this value (2.7. - 44.47 g; 2.5. - 43.56 g; 2.6. - 40.58 g; 2.4. - 39.65 g and 2.8. - 38.61 g). A mean variability is also observed in what it concerns the weight (11.39%).

Table 3. Main quantitative characteristics of the seeds V_2

V_2	100 seeds mass (g)	Seeds dimensions (mm)			Length/ Width	Width/ Thickness
		Length	Width	Thickness		
2 Mt	54.67 a*	14.41 bc	8.98 a	6.35 a	1.60	1.42
2.1.	48.49 bc	14.59 ab	8.62 b	6.28 a	1.69	1.37
2.2.	51.29 ab	15.19 a	8.65 b	6.26 ab	1.76	1.38
2.3.	46.17 bc	12.90 d	8.70 ab	6.58 a	1.48	1.32
2.4.	39.65 de	12.65 d	8.47 bc	5.32 d	1.49	1.59
2.5.	43.56 cde	14.39 bc	8.27 cd	5.52 cd	1.74	1.50
2.6.	40.58 de	12.17 d	8.22 cd	5.85 bc	1.48	1.40
2.7.	44.47 cd	13.76 c	7.91 e	6.22 ab	1.74	1.27
2.8.	38.61 e	11.41 e	8.08 de	6.41 a	1.41	1.26
2.9.	47.48 bc	12.90 d	8.70 ab	6.45 a	1.48	1.35
Mean	**45.50**	**13.44**	**8.46**	**6.12**	**1.59**	**1.39**
Stand. var.	5.18	1.21	0.33	0.42	0.13	0.10
CV%	11.39	9.03	3.91	6.84	8.36	7.23
Max	54.67	15.19	8.98	6.58	1.76	1.59
Min	38.61	11.41	7.91	5.32	1.41	1.26
	LSD 5% = 5.2321 LSD 1% = 7.1755 LSD 0.1% = 9.7666	LSD 5% = 0.7235 LSD 1% = 0.9923 LSD 0.1% = 1.3506	LSD 5% = 0.2865 LSD 1% = 0.3929 LSD 0.1% = 0.5348	LSD 5% = 0.3930 LSD 1% = 0.5390 LSD 0.1% = 0.7336		

*Different letters between variants denote significant differences (Duncan's test, $p<0.05$).

The kidney shape (2.2., 2.4., 2.5. and 2.7.) with a weak degree of curvature predominates at V_2 (Table 4). The other biotypes had an elliptic (2Mt, 2.1. and 2.3.) or circular to elliptic (2.3., 2.6. and 2.9.) shape.

The main color of control variant (light reddish brown) was found at 2.1. 2.8. The other three biotypes (2.2., 2.5. and 2.9.) had various shades of beige as their main color.

The white color (Figure 9) was present at 2.6. as main color and at 2.7. as secondary color (on half of grain).

A strong veining and difference in color of caruncula (raphe) can be observed at 2.6.

Weight at V_3 (Table 5) had the lowest values at 3.7. (47.65 g) and the highest values at control variant – 1 Mt (60.45 g).

Table 4. Main qualitative characteristics of the seeds V_2

V_2	Seeds shape[1]	Color			Hilum ring	
		Main	Secondar	Shape of secondar	Intensity	Color
2 Mt	elliptic	L reddish brown	reddish brown	in streaks and in patches	very weak	brown
2.1.	elliptic	L reddish brown	D brown	in streaks and in patches	weak	brown
2.2.	kidney [2] w	beige	mauve	in streaks and in patches	weak	yellowish
2.3.	circular to elliptic	L brown	grey	in very fine in patches	medium	brown
2.4.	kidney w	L brown	brown	in streaks and in patches	strong	brown
2.5.	kidney w	beige	reddish brown grey	in patches in fine patches	strong	orange
2.6.	circular to elliptic	white	brown	above the micropyle	absent	–
2.7.	kidney w	café au lait	white	half of grain	medium	brown
2.8.	elliptic	L reddish brown	reddish brown	in streaks and in patches	absent	–
2.9.	circular to elliptic	beige	grey	in very fine patches	strong	orange

[1]Shape of median longitudinal section; L = light; D = dark.
[2]Degree of curvature for kidney - shaped only: weak (w), medium (m) and strong (s).

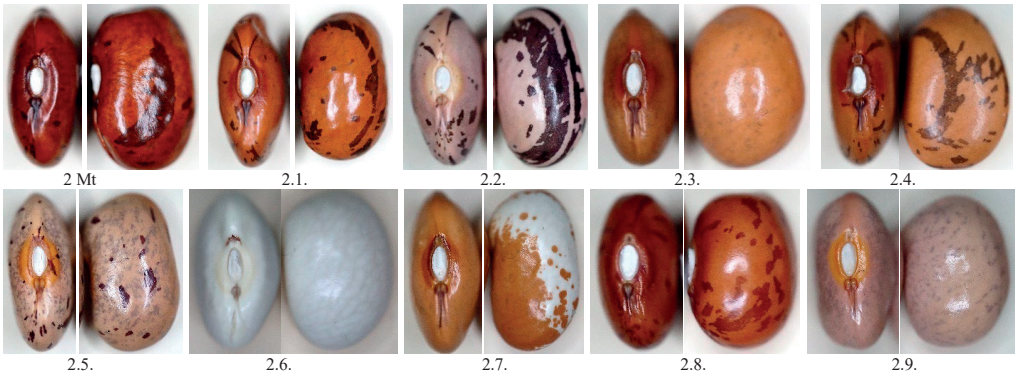


Figure 9. Aspects of qualitative characteristics of the seeds V_2

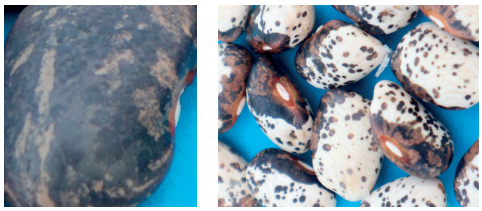
According to van Schoonhoven A. (1994), seeds belonging to this variant had a large size (more than 40 g). Seeds length registered the maximum value at 3.2 (16.76 mm) and the minimum value at 3.4. (13.36 mm). The coefficient of variation values for all parameters studied at V_3 were less than 10% (weight - 9.45% was the highest value), indicating low variability.

Table 5. Main quantitative characteristics of the seeds V_3

V_3	100 seeds mass (g)	Seeds dimensions (mm)			Length/ Width	Width/ Thickness
		Length	Width	Thickness		
3 Mt	60.45 a*	16.23 ab	8.45 b	6.13 c	1.92	1.38
3.1.	48.78 d	14.55 c	7.64 cd	6.07 cd	1.90	1.26
3.2.	60.35 a	16.76 a	8.93 a	6.29 c	1.88	1.42
3.3.	56.59 abc	13.64 d	8.67 ab	6.72 b	1.57	1.29
3.4.	53.56 bcd	13.36 d	8.38 b	7.37 a	1.59	1.14
3.5.	51.58 cd	16.44 ab	7.83 c	5.67 d	2.10	1.38
3.6.	58.92 ab	16.12 ab	8.45 b	5.68 d	1.91	1.49
3.7.	47.65 d	14.75 c	7.46 d	5.66 d	1.98	1.32
3.8.	49.11 d	14.08 cd	7.56 cd	6.16 c	1.86	1.23
3.9.	49.32 d	15.84 b	7.91 c	5.63 d	2.00	1.40
Mean	**53.63**	**15.18**	**8.13**	**6.14**	**1.87**	**1.33**
Stand. var.	5.07	1.25	0.51	0.56	0.17	0.10
CV%	9.45	8.21	6.29	9.09	8.92	7.84
Max	60.45	16.76	8.93	7.37	2.10	1.49
Min	47.65	13.36	7.46	5.63	1.57	1.14
	LSD 5% = 5.4448 LSD 1% = 7.4671 LSD 0.1% = 10.1636	LSD 5% = 0.7474 LSD 1% = 1.0250 LSD 0.1% = 1.3952	LSD 5% = 0.3346 LSD 1% = 0.4589 LSD 0.1% = 0.6246	LSD 5% = 0.4141 LSD 1% = 0.5679 LSD 0.1% = 0.7730		

*Different letters between variants denote significant differences (Duncan's test, $p<0.05$).

The most common shape to this variant (Table 6) was kidney (3 Mt, 3.1., 3.2., 3.5., 3.6., 3.9.), but it was met elliptical (3.3., 3.4.) and rectangular shape (3.7., 3.8.).
The combinations: main color beige, secondary color gray in patches (3.2.) or main color white, secondary colors brown and black on half the seed (3.3.) are much more suggestive in the field (Figure 10) that in the laboratory (Figure 11).



3.2. 3.3.

Figure 10. Aspects of seeds in the field

Table 6. Main qualitative characteristics of the seeds V_3

V_3	Seeds shape[1]	Color			Hilum ring	
		Main	Secondar	Shape of secondar	Intensity	Color
3 Mt	kidney[2] m	D brown	reddish brown	in patches	very weak	D brown
3.1.	kidney m	white	–	–	absent	–
3.2.	kidney m	beige	grey	in patches	medium	brown
3.3.	elliptic	white	brown and black	on half of grain	medium	brown
3.4.	elliptic	brown	black	in patches	absent	–
3.5.	kidney m	D beige	grey	in very fine patches	medium	reddish brown
3.6.	kidney s	brown	grey	in fine in patches	weak	brown
3.7.	rectangular	D mauve	–	–	absent	–
3.8.	rectangular	D reddish brown	L brown	in patches	absent	–
3.9.	kidney s	grayish brown	–	–	medium	L reddish brown

[1]Shape of median longitudinal section; L = light; D = dark.
[2]Degree of curvature for kidney - shaped only: weak (w), medium (m) and strong (s).



3 Mt 3.1. 3.2. 3.3. 3.4.
3.5. 3.6. 3.7. 3.8. 3.9.

Figure 11. Aspects of qualitative characteristics of the seeds V_3

CONCLUSIONS

In what it concerns weight of the seeds the maximum values were registered at control variants.
Various intensities of anthocyanin coloration of the hypocotyl show that the sown seeds were uniform only from a phenotypic point of view.
Under these conditions, the percentage of cross-pollination determined by insects cannot be specified.
A complete phenotypic characterization of bean seeds must include their quantitative and qualitative evaluation.
All biotypes will be cultivated to determine the characteristics of the plant. Only those that meet the breeding objectives will be kept and introduced into germplasm collection.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

FUTURE VISIONS OF INTEGRATED PEST MANAGEMENT (IPM) FOR MAIZE CROPS IN ROMANIA

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Abstract

Integrated pest management (IPM) has the potential to help farmers minimize the use of crop protection chemicals, relying primarily on preventive measures, reducing costs, and contributing to the transition to sustainable food systems. Although IPM approaches have been developed for a wide variety of crops and contexts, their adoption by farmers remains low across Europe. Previous studies show that farmers prefer a certain agricultural system due to socio-economic dependencies or support and support policies, market or conditions related to equipment and infrastructure, farm machinery and equipment, or insufficient professional training. This suggests that many farmers cannot simply adopt IPM and that changes and clarifications are needed along the entire chain from production, distribution, processing, market, and consumption to agricultural policy levels. This paper presents some of the results obtained to contribute to a greater extent to the co-creation of policies and actions together with the actors involved, regarding consumer health, plant and animal welfare, conservation and protection of the environment and biodiversity, adaptation of plant cultivation technologies to the effects of climate change.

Key words*: Integrated Pest Management, IPM, maize, future vision, conventional farming, organic farming.*

INTRODUCTION

Current EU legislation, such as the Sustainable Use of Pesticides Directive (SUD) and the European Green Deal, are part of a wider strategy to reduce reliance on chemical pesticides for agriculture production. Integrated Pest Management (IPM) emerged as a pest control framework promoting sustainable intensification of agriculture, by adopting a combined strategy to reduce reliance on chemical pesticides while improving crop productivity and ecosystem health (Zhou et al., 2024). Although IPM approaches have been developed for a wide diversity of crops and contexts, their uptake by farmers remains low across Europe (https://he-support.eu/). Earlier studies show that farmers sometimes feel stuck in a specific farming system due to economic dependencies and the lack of collectively sharing transition risks, among other issues (Hoes et al., 2023; Meuwissen et al., 2020; Siebrecht, 2020; Vermunt et al., 2022; Vrolijk et al., 2020). This suggests that many farmers cannot simply adopt IPM, necessitating changes to be made at the supply chain (processing, distribution, and consumption) and policy levels as well (Hoes, 2024).

Intense long-term utilization of pesticides on cultivated area causes substantial environmental and health concerns (Aktar et al., 2009). As a pollutant, when applied on ground, pesticides are able to disturb the soil properties because of its contaminant effect and harmful impact on biodiversity. Over entire pesticides utilized for crops, it has been stated that an extremely small percent reaches the target organism whereas the residues of it pollute the surrounding field (air, water, and ground). All these aspects require a permanent monitoring in order to avoid a series of imminent dangers (Dragomir Bălănică et al.,

2024). Adopting good agricultural practices, especially on the soil management, is a key factor in granting food, clean water, feed, energy, safe climate, diverse ecosystem services and biodiversity etc. for future generations (Musat et al., 2021).
From the point of view of farmers' attitude towards these challenges, sometimes there are certain limits in reducing the application of pesticides in agriculture (Chèze et al., 2020), and as a perspective it is difficult to obtain food to feed more than 9 billion people until 2050, without the application of these products. Plant protection products are a substantial part of modern day agriculture (Ganchev, 2022).
On the other hand, maize crops around the world are an important source of food production for both humans and animals (Erenstein et al., 2022), and also in Romania, where the surface oscillates annually between 2.1-2.5 million ha. This is the reason why, in the case of the Support project (Supporting Uptake Integrated Pest Management and Low-Risk Pesticide Use) (https://he-support.eu/), within the Horizon Europa program (HORIZON-CL6-2022-FARM2FORK-01), for Romania the assigned National Cluster Crop is maize for the analysis of current and future IPM practices.

MATERIALS AND METHODS

This study presents the opinion of farmers and other target groups (researchers, input suppliers, consultants, decision-makers, etc.), regarding the perspectives and future visions of the implementation and adoption of IPM strategies for maize crops in Romania.
Two co-creation workshops took place as part of Support project activities in Romania, organised by University of Agronomic Sciences and Veterinary Medicine of Bucharest (USAMV of Bucharest) and the Forum of Professional Farmers and Processors from Romania (FAPPR), partners within this project. These co-creation workshops applied a backcasting approach (Hoes, 2024). Backcasting involves developing a desirable future vision and exploring which changes are needed in the present to move closer towards this goal (Vergragt & Quist, 2011).
50 people participated in these actions, including farmers, researchers, consultants, experts, distributors of agricultural inputs, authorities.
The two working groups drew up the future visions of IPM in maize crops, both for the conventional system and for the organic agriculture system (Figure 1).

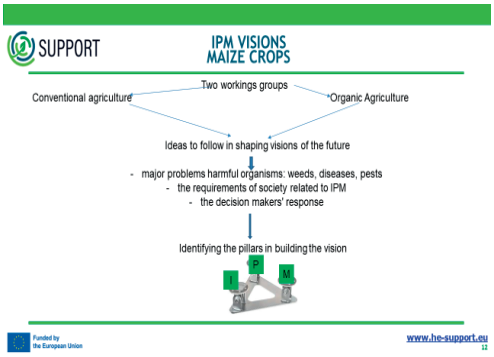


Figure 1. Methodology of the workshops organization

The answers were followed through the prism of three dimensions, namely: major problems due to harmful organisms, weeds, diseases and pests of maize crops; the requirements of society; the response of the authorities. Starting from the three dimensions, the pillars on which the IPM vision is to be based were finally identified.

RESULTS AND DISCUSSIONS

Farmers, policy makers and other actors need reliable data and information in order to make sound decisions about crop protection and crop protection policy. This requires a clear understanding of the existing crop protection practices in different crops across the EU, as well as their environmental, social and economic impacts. This also necessitates an overview of potential IPM practices that can be applied in different crops and regions and the potential gains in terms of the environmental, social and economic impacts (https://he-support.eu/).
IPM can include modern agricultural technologies, communication tools based on sustainability principles, but also on traditional methods, integrating human, environmental, social and economic factors in food production (Xu et al., 2024).

Farmers' access to alternative methods and lower-risk products could help them apply Integrated Pest Management in maize crops (https://op.europa.eu/webpub/eca/special-reports/pesticides-5-2020/en/).
The Agricultural Policy can help support the sustainable use of plant protection products, for example through financial support for measures such as organic farming and environmental schemes (https://agriculture.ec.europa.eu/sustainability/environmental-sustainability/cap-and-environment_en).
Facilitating the use of alternative methods and access to lower-risk products can help support a more sustainable pest management process (https://www.eca.europa.eu/lists/ecadocuments/sr20_05/sr_pesticides_en.pdf).
Research and innovation activities are important to ensure that alternative pest management methods and low-risk products are available to enable the implementation of IPM (https://www.eca.europa.eu/lists/ecadocuments/sr20_05/sr_pesticides_en.pdf).

The vision of IPM in the maize crop in conventional farming system, in Romania
According to current statistics, the maize crop in Romania continues to be of major importance, even if in recent years, due to less favourable climatic conditions, the maize production has suffered greatly from drought.
This has led to a reduction in total production, from over 14.8 million tonnes to only 8 million tonnes (Table 1).

Table 1. Current status of maize crops in Romania (MADR, 2024)

Year	Area (ha)	Average production (kg/ha)	Total production (tons)
2015	2,605,165	3,462	9,021,403
2016	2,580,975	4,159	10,746,387
2017	2,402,082	5,959	14,326,097
2018	2,439,842	7,644	18,663,939
2019	2,678,504	6,502	17,432,223
2020	2,537,104	3,977	10,096,689
2021	2,549,281	5,802	14,820,693
2022	2,431,106	3,298	8,037,134
2023	2,195,344	3,982	8,743,995

From the point of view of problems due to harmful organisms, the following can be summarized:

- In the future, maize crop will be greatly influenced by climatic conditions, and drought can be a limiting factor, both in terms of production and its quality, increasing the variability in time and space of maize production.
- In drought-affected areas, farmers who will not be able to irrigate will reduce their maize area or stop growing maize, while farmers who will be able to irrigate will increase their maize area, which will be of interest from the economic point of view.
- The tendency will be to concentrate the cultivation of maize in the areas where it will be irrigated. As a result, maize hybrids more tolerant to climatic conditions will be obtained.
- Due to the fact that some pesticides will disappear from the market, harmful organisms will develop resistant breeds, and there is a risk that the accepted pesticide products will no longer have the same effectiveness, while they will have higher costs.
- The number of control treatments will depend on the virulence with which the maize plants will be attacked.
- Maize will be a profitable crop, through the range of products resulting from its processing.
- New technologies will be more expensive, and the implementation cost will be higher and this will be reflected in the price of the final product.
- The effect of different tillage options, with positive and negative effects on harmful organisms, must be taken into account.
- Thus, in the variants of minimal tillage and the variant without tillage there can be a lower degree of weeding through the germination of seeds, seeds that remain on the surface of the soil, without being incorporated into the soil where they can germinate and give rise to weeds, while the species of perennial weeds can proliferate. Also in these systems, maize plants can be more exposed to disease and pest attack (for example, the attack of rodents is intensified, especially when the soil is no longer tilled or the entire surface is no longer tilled or in depth).
- Drip irrigation, mulching will be widely adopted.

From the point of view of society's requirements, the following can be summarized:

- Society sees pesticides as something bad. It also tends to place the onus entirely on farmers, without understanding and accepting that reducing pesticide use will come with a number of additional costs to farmers, which will mean more expensive final products.
- Society will want cheap products that will provide daily food. As such, social perception will have to be improved, in this sense, the mass media having an important role, having to provide correct information to society.

From the point of view of the answers of the authorities, the following can be summarized:

- From a legislative point of view, new, stricter rules will be imposed on the use of pesticides.
- These regulations are perceived to be increasingly less farmer friendly. There is a need for clearer and more accurate official communication.
- Technologies that are gentler on the environment will continue to be supported.

The main constraints and opportunities for applying IPM to maize crops in Romania are presented in Figures 2 and 3.

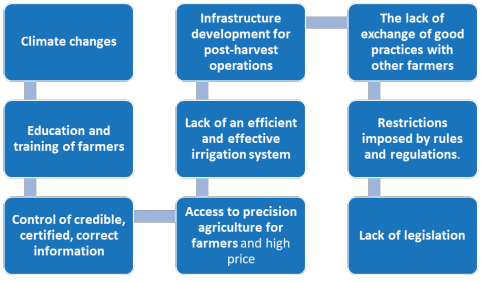


Figure 2. Main constraints for applying IPM to maize crops in Romania

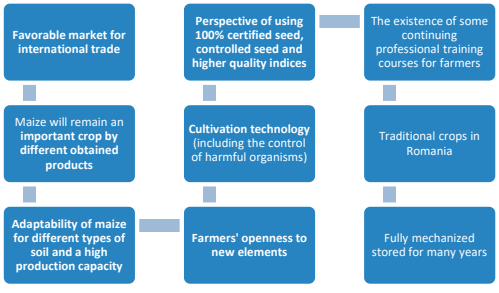


Figure 3. Main opportunities for applying IPM to maize crops in Romania

The vision of IPM in maize crop in organic farming.

In the organic farming, strict rules must be followed regarding the use of plant protection products. As a result, the control over IPM will largely depend on the rules imposed by the related legislation.

According to FiBL statistics, of the 579 thousand ha occupied by agricultural land managed in an organic system in Romania, over 35.5 thousand ha are cultivated with maize.

Table 2. Current area of organic maize crops in Romania (https://statistics.fibl.org/, 2024)

Year	Area (ha)	Maize area (ha)
2015	245,924.00	23,137.00
2016	226,309.00	16,643.00
2017	258,471.00	19,671.00
2018	326,260.00	26,745.00
2019	395,228.00	36,720.00
2020	468,887.00	40,164.00
2021	578,718.00	35,576.00
2022	644,520.00	35,576.00

From the point of view of problems due to harmful organisms, the following can be summarized:

- It is believed that due to future climate changes and global warming, maize hybrids will appear more tolerant and resistant to environmental conditions, as well as to the attack of harmful organisms.
- Of course, there will be the risk of the appearance of new organisms harmful to maize, with a much greater intensity and frequency of attack. As a result, the new technology will be based on the use of precision agriculture, drones and robots, which will help to predict the action of harmful organisms, and through information systems, IT, technology, farmers will be instantly warned by phone about the risk of the appearance of a pest or pathogen. They will know exactly when to act and will use small amounts of biopesticides, which will contribute to ensuring the economic efficiency of the farmers' activity, which will protect the health of consumers and the environment.
- The soils will be balanced from a microbiological point of view, based on conversion periods that will be more effective

both in terms of duration and intensity for the transition from conventional to organic farming. This will translate into lower risks due to harmful organisms.
- The personnel who will work in precision agriculture will need to be trained and trained, and funds will be needed that will be used for this purpose.
- Plant protection products will be based more on plant physiology and will be applied exactly when harmful organisms are most sensitive.
- Drip irrigation, mulching will be widely adopted.
- There will be databases in the mother tongue, which will be made available to farmers to better know the biology and the way of attack and methods of controlling pests, diseases and weeds so that the best method suitable for the concrete conditions of the respective farm.
- The products allowed in organic agriculture to control weeds will remain a problem for the maize crops, having limits imposed by the legislation.

From the point of view of society's requirements, the following can be summarized:
- The consumers will continue to appreciate organic maize products. However, the price will remain high for these products. Throughout the chain, these products will have to be promoted, and education and professional training will have a major role.
- Legislation and control will have to take into account all actors in the chain, so as to improve consumer confidence in this product.
- The safety and traceability will guarantee the facilitation of the export of products.

From the point of view of the answers of the authorities, the following can be summarized:
- The authorities will have to respond to the demands of society.
- The safety of traceability must be ensured by legislation and product controls throughout the chain.

Farmers, policy makers and actors need reliable data and information in order to make sound decisions about crop protection and crop protection policy. This requires a clear understanding of the existing crop protection practices in different crops across the EU, as well as their environmental, social and economic impacts.

The main constraints and opportunities for applying IPM to organic maize cultivation in Romania are presented in Figures 4 and 5.

This also necessitates an overview of potential IPM practices that can be applied in different crops and regions and the potential gains in terms of the environmental, social and economic impacts. Support project proposes the application of indicators reflecting the economic, social and environmental impacts of crop protection, which enables them to make their crop protection more sustainable.

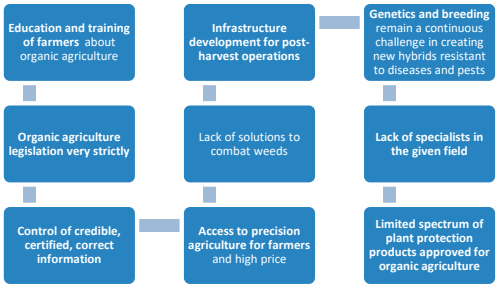


Figure 4. Main constraints for applying IPM to organic maize crops in Romania

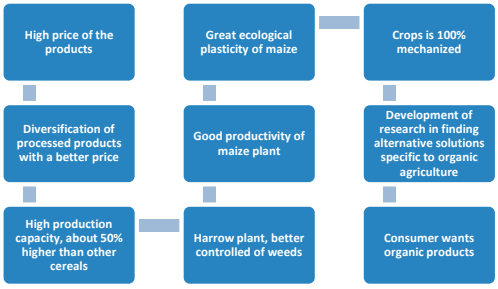


Figure 5. Main oportunities for applying IPM to organic maize crops in Romania

CONCLUSIONS

The health of crops and soil, of the environment in general, is threatened by numerous harmful species that can represent a major risk for agricultural production, for food security and safety at the global level.

Integrated measures to prevent and preserve crop health and Integrated Pest Management (IPM) have become an important topic in agricultural policies worldwide, in Europe, but also in Romania.

IPM can include modern agricultural technologies, communication tools based on sustainability principles, but also on traditional methods, integrating human, environmental, social and economic factors in food production. Consumers will continue to want safe products, without residues, which is why a stable and fair market will be developed for the sale of these products.

ACKNOWLEDGEMENTS

This article was financed by the Faculty of Agriculture, University of Agronomic Sciences and Veterinary Medicine of Bucharest and by the "SUPPORT project - Supporting Uptake Integrated Pest Management and low-risk pesticide use" project, Horizon Europe, ID:101084527.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

EVALUATION OF TALL FESCUE (*Festuca arundinacea*) GENETIC RESOURCES FOR BREEDING ACTIVITY

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Abstract

The breeding program involves new objectives and approaches for adapting to changing climatic conditions by creating productive varieties with increased tolerance to drought. The aim of research is the evaluation of the germplasm through observations and measurements to choose valuable resources of initial breeding material. Were analyzed 20 accessions. The studied genotypes can be grouped into three categories from precocity point of view, difference between the earliest variety, Kerestelny (28.04) and the latest, Luna (25.05) was 27 days. The abundance of vegetative shoots was combined with the abundance of generative shoots, to break the existing negative correlations between seed production and DM production. Regarding plant height a large inter-varietal and intra-varietal variability was found, with average values above 100 cm in the varieties: Krasnodarska 115.2 cm and with values below 50 cm in the Luna variety 48.5 cm. The most resistant genotypes to Puccinia.sp. with the frequency of over 80% resistant plants are: Luna: 90%, Bronson: 89.5%, Prolate 86.7% and the most sensitive are Szarkad 5: 20% and Bull 20.5% unattached.

Key words*: disease resistance, genotype, generative shoots, plant height, tall fescue, vegetative shoot.*

INTRODUCTION

The tall fescue is a perennial grass with an extremely complex biology, characterized by good adaptation both in conditions of excess moisture and drought. At the same time, it grows normally on acid soils and on salty soils (Moga I. and Schitea Maria, 2000; Popa et al., 2008).
It withstands high temperatures, but also low ones, as well as periods of prolonged drought, thanks to the more developed and deeper root system compared to other perennial grasses, favoring the use of water and nutrients from the deeper layers of the soil and at the same time preventing soil compaction (Mocanu et al., 2021).
In Romania, tall fescue has been introduced into culture in the last 25 years, but the current breeding program involves new objectives for adapting to changing climatic conditions and reducing the impact on the environment by creating productive varieties with increased tolerance to drought.
The success in plant breeding, obtaining new genotypes, superior to the existing ones and adapted to the new environmental conditions, depends on the wealth of valuable genes and the genetic diversity of the germplasm.
In general, the selection applied to allogamous species leads to the achievement of genotypes differentiated from the initial forms (not subject to the selection process), as a result of the continuous recombination of genes in each generation (Savatti et al, 2004).
The aim of this research is the evaluation of the germplasm in the selection field through observations, measurements and determinations in order to know the morphological and physiological characteristics of the genotypes to choose valuable sources of initial material.

MATERIALS AND METHODS

In the selection field within the breeding laboratory of the Grassland Research and Development Institute - Brașov, 20 tall fescue genotypes of different eco-geographic origin were used as research material. These were planted individually in the field in autumn 2023, 10 plants per row of 5 rows. Appreciated number of generative and vegetative shoots, with notes from 1 to 5, carried out on a scale of 5 steps: 1 - very few ... 5 - very many, and the assessment of the degree of rust attack (*Puccinia graminis* and *Puccinia striiformis*) was carried out on the scale of 5 steps: 1 - 0% … 5 - >100% of the leaf surface. Measurements were made on the plant's height, the heading date and observations were made on the form of the plant

(L - lax, S - semi-erect, E - erect). All information was collected from the field between April and July 2024.

The purpose of these observations and measurements is to identify the most valuable genotypes that could be the basis for the creation of new qualitatively and quantitatively superior synthetic combinations and the highlighting and selection of the parental forms of *Festuca arundinacea*, which meet the breeding objectives: increasing the forage and seed production, disease resistance, fineness of leaves and method of usage.

The meteorological conditions of the years 2023 and 2024 (Table 1) had positive deviations, compared to the multiannual average from the point of view of temperatures, and negative deviations, from the point of view of precipitation. Average annual temperatures were higher than the multiannual average with 2.3°C, respectively 3.6°C.

Table 1. Meteorological conditions from Brașov stationary, 2023-2024

Years	Annual average I-XII	Deviation	Vegetation period IV-IX	Deviation during vegetation period
Temperature (°C)				
2023	10.1	+2.3	14.8	+0.6
2024	11.4	+3.6	17.6	+2.7
Average 59 years	7.8	0	14.2	0
Precipitation (mm)				
2023	570.4	-182.8	407.8	-121.3
2024	730.6	-22.6	485.9	- 43.2
Average 25 years	753.2	0	529.1	0

From the point of view of precipitation, the year 2023 recorded a deficit of -182.8 mmm compared to the multiannual average, and in the year 2024 a deficit of -22.6 mm was recorded. During the vegetation period, there was a deficit of precipitation, in both studied agricultural years. The plants were irrigated only in 2023.

The recorded data were statistically processed in the Statistica 7 software package, using ANOVA, corelation , principal component and cluster analysis.

RESULTS AND DISCUSSIONS

Regarding the heading date, the analysed genotypes can be grouped into three categories: early (28.04 - 6.05), intermediate (7.05 - 15.05) and late (16.05 - 25.05), allowing the selection of genotypes for the creation of varieties with different earliness. The difference between the earliest variety, Kerestelny (28.04) and the latest, Luna (25.05) was 27 days. To ensure the homogeneity of the variety, phenotypically similar plants were chosen. The abundance of vegetative shoots determines the forage production, the main criterion for evaluating agronomic performance in order to certify a variety, grades of 5 and 4 indicating a rich foliage. By combining with the abundance of generative shoots (along with the other elements of fruiting: number of seeds / inflorescence, MMB) it is aimed to create varieties that can put a high biological potential of fruiting and breaking the existing negative correlations between seed production and forage production. Plant height, correlated with the abundance of vegetative shoots, is a selection criterion for forage production as well as for use. A large inter-varietal as well as intra-varietal variability was found, with average values above 100 cm in the varieties: Krasnodarska: 115.2 cm, Terros and Visuki: 104 and 103.7 cm, respectively, and with values below 60 cm in the Prolate varieties: 57 cm, Luna: 48.5 cm and Jotvincs: 53.5 cm.

Disease resistance is an essential factor in obtaining qualitatively and quantitatively high forage production without negative impact on seed quantity and quality.

Among the diseases specific to perennial grasses, rust, transmitted by *Puccinia* sp., is the most damaging, both in terms of frequency and intensity. The attack frequency notes were made on each plant in the selection field, in order to select resistant genotypes, the table showing the average attack on each genotype. The most resistant genotypes, with the frequency of over 80% resistant plants are: Luna: 90%, Bronson: 89.5%, Prolate 86.7% and the most sensitive are Szarkad 5: 20%, Bull 0.5% and Terros II: 30% unattacked plants (Table 2).

The results obtained from the observations and biometry (genotypes) from the selection fields were processed statistically, through several tests, to validate the respective results. The graphical representation of the correlation relationships ensured with a statistical significance by the Pearson correlation can be found in the following graph (Figure 1).

Table 2. Morphological observations in the selection field

Genotype	Heading date	P. form	V. shoot	G. shoot	Plant H (cm)	Dis. resist (%)	Use
Szeveleny 4	1.05	SE	**4**	4	92.7	50.0	M
Albena	8.05	L	**4**	3	89.0	41.0	M
Kereteleny	28.04	SE	**4**	4	89.5	32.5	M
Szarkad 5	2.05	E	**3**	4	96.7	20.0	M
Bull 2021	3.05	SE	**4**	4	97.0	20.5	M
Proba	6.05	SE	**5**	5	98.7	67.5	M
Koreta	12.05	L	**5**	3	80.5	87.2	G
Bronson	6.05	E	**5**	5	100.5	89.5	M
Fawn	5.05	SE	**4**	4	99.7	73.7	M
Prolate	20.05	L	**5**	4	57.0	86.7	G
Luna	25.05	L	**3**	3	48.5	90.0	G
Adela 2021	10.05	SE	**4**	4	67.0	77.5	M
5.. - 6..	12.05	SE	**4**	4	67.0	66.7	M
Terros I	10.05	SE	**4**	4	89.7	60.0	M
Vio Jucu	10.05	SE	**3**	4	97.7	60.0	M
Terros II	10.05	SE	**4**	4	104.0	30.0	M
Krasnodarska	5.05	SE	**4**	5	115.2	37.5	M
Visuki	10.05	SE	**4**	4	103.7	53.3	M
Jotvincis	24.05	L	**5**	3	53.5	50.0	G
Grebalovska	15.05	L	**4**	3	60.2	70.0	G

V. shoot = vegetative shoots.: 13.05.2024 - 1 - very few; 5 - very many
G. shoot = generative shoots: 30.05.2024 - 1 - very few; 5 - very many
Dis. resist.= disease resistance: 03.08.2024 – 1 - 0% attack; 9 - 100% attack
Use = Usage mode : M - mixt, G - turf,
P. Form = Plant form - L - lax, S - semi-erect, E - erect
Plant H = Plant heigh

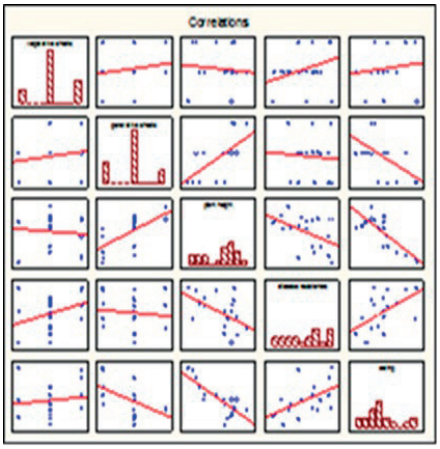


Figure 1. Graphic representation of correlation relations

In the graphic representation is the expression of the correlation between the five studied characters.

On a general analysis of all the graphs, several genotypes stand out as the best, depending on the analyzed character.

In Table 3, the correlation coefficient highlights the following statistically ensured interdependencies in the expression of morphoproductive characters in genotypes selected from the selection fields: the height of the plant shows a distinctly significant positive correlation (0.65) with generative shoots and significantly negative with disease resistance (-0.49) and very significantly negative with the heading date (-0.79).

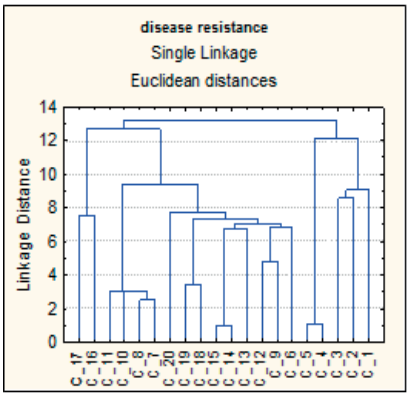
Generative shoots was correlate significantly negatively (-0.56) with the heading date, while disease resistance correlates significantly with the heading date(0.59).

The morphoproductive characters investigated in the selection and breeding process of *Festuca arundinacea* were analyzed through the correlation matrix to estimate the correlation of the analyzed genotypes.

Also, through this procedure, the null hypothesis that the population correlation between pairs of variables is equal to 0 was tested and statistically invalidated.

Table 3. Correlation matrix

	Vegetative shoots	Generative shoots	Plant H. (cm)	Disease resistance	Heading
Vegetative shoots	1.00	0.15	-0.10	0.34	0.13
Generative shoots		1.00	**0.65****	-0.10	**-0.56** **
Plant H (cm)			1.00	**-0.49***	**-0.79** ***
Disease resistance				1.00	**0.53***
heading date					1.00
$r_{5\%} = 0.44$; $r_{1\%} = 0.55$; $r_{0.1\%} = 0.67$					



C1-Szeveleny 4, C2-Albena, C3-Kereteleny, C4-Szarkad 5, C5-Bull 2021, C6-Proba, C7-Koreta, C8-Bronson, C9-Fawn, C10-Prolate, C11-Luna, C12-Adela 2021, C13- 5.. - 6, C14-Terros I, C15-Vio Jucu, C16-Terros II, C17-Krasnodarska, C18-Visuki, C19-Jotvincis, C20-Grebalovska

Figure 2. The dendrogram for the representation of disease resistance

Studying the dendrogram of the hierarchical cluster analysis performed on the 20 studied genotypes (Figure 2), we can conclude that they are grouped according to the rust resistance of each one. Thus, two cluster groups are formed, with great similarity, the first being made up of

genotypes C 1-4 (Szeveleny 4, Albena,, Kereteleny, Szarkad 5), and the second with the other 16 genotypes.
It is important that when choosing the plants that will be selected to continue the breeding process, there are phenotypically similar plants with high disease resistance.
They will not choose phenotypically similar plants, and with high productive capacity, but with low disease resistance.
Following the calculations made through the principal components analyses (PCA), two components was resulted, so the first 2 components bring a variance of 78.77%.
It is found that, accepting the expression of the initial causal space, respectively of the variables under study, through a single main component, only 52.75 of the initial variance is explained. Extending the number of main components to 2, the explanation of 78.77% of the total variance is ensured (Figure 3).

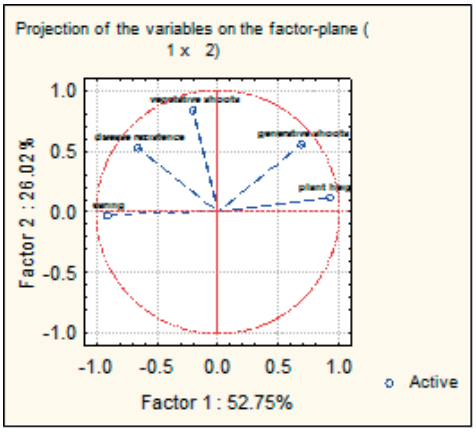
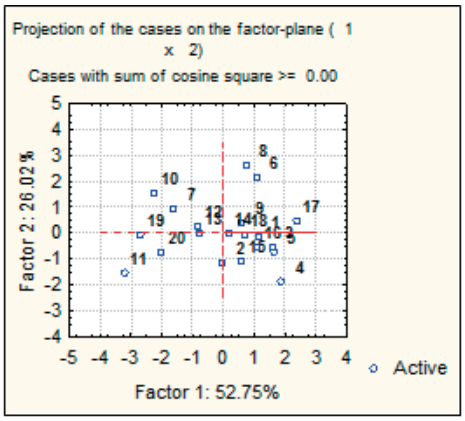


Figure 3. The projection of the variables in the plane of the factorial axes of the two main components (Factor 1/PC 1 and Factor 2/PC 2)

The varieties Krasnodarska, Jotvincis, Prolate, represented in figure 5 with points 17, 19 and 10, are very close to principal component 1 (PC1) namely for heading date, plant height, generative shoots and disease resistance. Bronson, Proba and Szarkad varieties are part of the principal component 2 (PC2) represented here by the morphoproductive character, vegetative shoots (Figure 4).
It is important that in the selection of the most valuable genotypes a balance between the analyzed characters is found, so that the future varieties have high productive potential both in the production of seeds and forage.



1-Szeveleny 4, 2-Albena, 3-Kereteleny, 4-Szarkad 5, 5-Bull 2021, 6-Proba, 7-Koreta, 8-Bronson, 9-Fawn, 10-Prolate , 11-Luna, 12-Adela 2021, 13- 5.. - 6.., 14-Terros I, 15-Vio Jucu, 16-Terros II, 17-Krasnodarska, 18-Visuki, 19-Jotvincis, 20-Grebalovska

Figure 4. The projection of the genotypes in the plane of the factorial axes of the two main components (Factor 1/PC 1 and Factor 2/PC 2)

The genetic variability of all traits important for breeding process represent a basic prerequisite for successful selection (Babic et al., 2018).
The increase in seed production is mainly determined by the number of generative shoots, therefore for the creation of new varieties with a high potential for producing the amount of seeds it is important that in the breeding process the genotypes that produce many generative shoots are selected.
Forage production and its quality is determined by a high percentage of leaves, which imparts better quality and palatability to the forage. Therefore, one of the important breeding objectives is to increase the number of leaves and the size of the leaves in the improved varieties.
Obtaining products with the highest quality and very good quality depends to a large extent on the species and variety used.
The introduction, expansion, as well as the maintenance of valuable varieties in the culture depend to a great extent on the culture conditions, on the economic requirements, but above all on the ecological resources, to which a new genotype must have a high degree of adaptability (Varga et al., 1998)
Principal component 1 (PC1) which represented 52.75% of the total variation, includes the

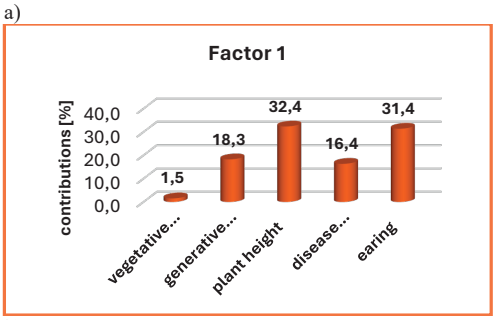
variables with the highest correlation coefficients: Height - a very tight, positive correlation (r=0.92), height - a very tight, negative correlation (r=-0.91), Generative shoots – a close correlation (r=0.69), positive correlation and rust resistance a correlation tight (r=-0.66), negative correlation

Principal component 2 (PC2) which represented 26.02% of the total variation includes: vegetative shoots, a very tight correlation (r=0.84), generative shoots (r=0.55), plant height (r=0.12), rust resistance (r=0.53) and tillering (r= -0.03) (Table 4).

Table 4. Factor-variable correlations, based on correlations

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
V. shoots	**-0.202077**	**0.835957**	0.509863	-0.017747	0.008034
G. shoots	0.694431	0.553942	-0.318648	0.309148	-0.117493
P. height	0.924317	0.116685	-0.027679	-0.087551	0.351555
Disease resistance	-0.657033	0.529875	-0.467556	-0.258730	0.044614
Heading date	-0.910289	-0.026965	-0.046903	0.337626	0.233357

a)



b)

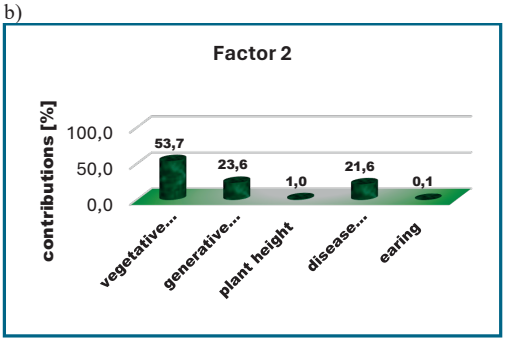


Figure 5. Principal Component Analysis: PCA 1 (a) and PCA 2 (b)

Within PCA 1, the biggest contribution is the height of the plant (32.4%) and the heading date (31.4%), and the number of vegetative shoots influences only 1.5% (Figure 5a).

Within PCA 2, vegetative shoots have the largest contribution, representing 83.7% (Figure 5b).

CONCLUSIONS

The abundance of vegetative shoots combining with the abundance of generative give a high biological potential for break the existing negative correlations between seed production and forage production.

The plant height, correlated with the abundance of vegetative shoots, is a selection criterion for forage production as well as for use. A large inter-varietal as well as intra-varietal variability was found, with average values above 100 cm .

For the selectioned plants, it is important to meet the new breeding objectives, namely: adaptability, perennially, productivity and resistance to diseases and pests, under the conditions of climate change.

The selected genotypes represent valuable material for the development of high-yielding, stress-tolerant cultivars.

The strict choice from the selection field of the most valuable parental forms can lead to high chances of success in order to create synthetic varieties of perennial grasses.

Following the analysis, the following genotypes were selected for polycross breeding activity, in the next year, tall plants from the genotypes with a large number of vegetative and generative shoots with resistant to diseases.

ACKNOWLEDGEMENTS

This research work was carried out with the support of Grassland Research and Development Institute - Brașov and also was financed from Project ADER 15.1.1 “Research on obtaining new forage plant varieties in perennial grass species: *Festuca arundinacea, Festuca pratensis, Festuca rubra, Dactylis glomerata, Lolium perenne, Phleum pratense, Bromus inermis* and perennial legumes for grasslands: *Trifolium repens, Lotus corniculatus, Onobrychis viciifolia,* adapted to climate change to improve animal feed and create new possibilities of ecological reconstruction by greening degraded lands”.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

RELATIONSHIPS OF NDVI AND CHLOROPHYLL CONTENT WITH YIELD COMPONENTS OF DIFFERENT ACCESSIONS OF *Phaseolus vulgaris* L. AND VIGNA (*Vigna unguiculata* L. Walp.)

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Abstract

The aim of this study was to investigate the relationship between NDVI, Chlorophyll and Nitrogen content in leaves with yield components of different Phaseolus vulgaris L. and Vigna (Vigna unguiculata L. Walp.) genotypes. Field experiment was conducted in 2024 year in the experimental field in Institute of Plant Genetic Resources, Sadovo, Bulgaria. The normalized difference vegetation index (NDVI), leaf chlorophyll content (Multy pigmentometer MPM - 100) and Nitrogen content (N-Pen) were measured on leaves directly in the field. The various plant characteristics in addition were measured such as plant height, plant weight, plant weight without pods, pod length, number of pods per plant, number of beans per pod, weight of beans per plant of cowpea (Vigna unguiculata L. Walp.) and common beans (Phaseolus vulgaris L). It was found that NDVI index has positive significant correlation with Chlorophyll (r = 0.69) and higher between N and chlorophyll (r = 0.75). It was also found the morphological traits studied characteristics as days to flowering, plant weight, number of seeds in pod showed higher correlation with chlorophyll index in compare to NDVI and N content.

Key words*: NDVI, chlorophyll, cowpea, yield.*

INTRODUCTION

There is a growing interest in the search for rapid, non-destructive and efficient methods to assess plant physiological and biophysical parameters, plant health and food quality (Tores et al., 2020). Different types of vegetative indices based on spectral information in the visible and near-infrared regions are being compiled and searched for in order to monitor vegetation quickly and easily remotely such as normalized vegetative index (NDVI), Chlorophyll Absorption in reflectance index (CARI), Chlorophyll Index at red edge (Cl green), Pigment index (PI), Photochemical reflectance index (PRI) (Veleva et al., 2022; Weiss et al., 2004) NDVI has also been shown to be related to a number of other plant parameters such as leaf area index (LAI), biomass, photosynthetic activity (Mahlein et al., 2013; Mahlein et al., 2012; Baghzouz et al., 2010; Bell et al., 2004). Leaf chlorophyll content is an important physiological parameter that can serve as an indicator of nutritional status, plant stress or senescence. The amount of chlorophyll and nitrogen in leaves provides extremely valuable information about plants, the parameters of which have been used to assess the total photosynthetic capacity and productivity of plants. Within precision agriculture, data from different vegetation indices are used to monitor the vegetation process during different seasons. Rapid estimation of chlorophyll and nitrogen content in foliage is critical for determining the optimum fertilization requirement of crops to improve the efficiency of precision agriculture (Haboudane et al., 2004). *Phaseolus vulgaris* L. and Vigna (*Vigna unguiculata* L. Walp.) are grain legumes, high protein crops, and beans are one of the main traditional legumes on our table in our country and in the world. (Abebe & Alemayehu, 2022; Hall et al., 2003) *Vigna unguiculata* L. Walp. as known as cowpea is characterised by smaller seeds, a rough or smoother surface and a well coloured hilum. Vigna is known for its tolerance to high temperatures and drought, characterising it as highly adaptable to global climate change with pronounced warming and drying during the growing months (Tzanova et al., 2023; Barros et al., 2021).

Although spectral reflectance indices have been used to assess yield in cereals such as wheat,

barley, rice information on the performance of these indices for bean and cowpea is needed.

MATERIALS AND METHODS

Field experiment was conducted in the experimental field in Institute of Plant Genetic Resources, Sadovo, Bulgaria in 2024 year. 24 genotypes of cowpea (*Vigna unguiculata* L. Walp.) and 20 common bean (*Ph. vulgaris* L.) with different geographical origins were included in the study (Tsanova et al., 2023). Sowing was carried out manually, with a row spacing of 70 cm, an intra-row spacing of 5 to 10 cm depending on the seed size, a sowing depth of 5-6 cm (bean) and 4-5 cm (cowpea). The studied legumes were grown without irrigation. Weed control is brought out by several hand hoeing of the row spacing and weeding inside the row. Harvesting of beans and vigna was done manually at the stage of full maturity of the beans (90-95%). At the beginning of the growing season, a representative plant was marked from the germinated plants for each of the genotypes studied. The normalized difference vegetation index (NDVI) with Plant Pen 310 (PSI, Czech Republic), leaf chlorophyll content (Multy pigmentometer MPM - 100) and Nitrogen content (N-Pen, PSI, Czech Republic) were foliar measured directly in the field. (Figures 1 and 2) Field measurements of leaves from tagged plants were carried out at the following phenophases: pre-flowering, flowering and pod formation. The various plant characteristics in addition to grain yield were measured such as number of days to full flowering, plant height (cm), plant weight (g), plant weight without pods (g), pod length (cm), number of pods per plant, number of beans per plant, number of beans per pod, weight of beans per plant (g) of cowpea (*Vigna unguiculata* L. Walp.) and common beans *Phaseolus vulgaris* L.



Figure 1. Portable spectral device multipigmentometer MPM - 100 (Opti-science, USA)



Figure 2. Portable spectral device N-Pen (PSI - Photon Systems Instruments, Czech Republic)

RESULTS AND DISCUSSIONS

It was found that NDVI values between 0.33 and 0.77 for bean genotypes, with a mean value of 0.77. According to cowpea plants, NDVI values were between 0.62 and 0.77, with a mean of 0.73. The vegetative index varied very narrowly in the leaves of cowpea samples with SD = 0.03, in contrast to the bean samples with SD = 0.10. The histogram (Figure 3) presents a more detailed reading of the NDVI variations in the different phases of the studied legumes. The highest NDVI index was recorded during the pre-flowering period of both legumes, after which it started to decrease until pod formation, as NDVI decreased to 0.62 in cowpea leaves and twice as low as 0.33 for bean leaves. (Figure 4)

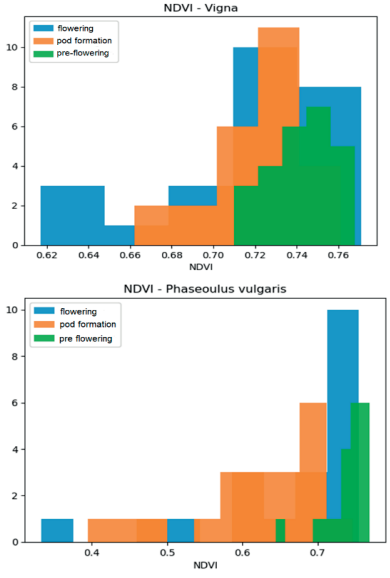


Figure 3. Histogram for NDVI in leaves of *Ph. Vulgaris* and *V. unguiculata*

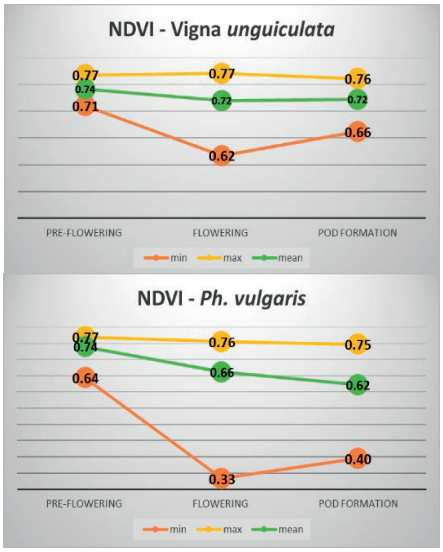


Figure 4. Trends of min., max. and mean NDVI values in leaves of *V. unguiculata* and *Ph. vulgaris*

Table 1. Discriptive statistics of yield components in *Phaseolus vulgaris* L. and *Vigna unguiculata*

Yield components	range	mean	SD
	Vigna unguiculata		
Days to full flowering	13-15	13.7	0.98
Plant height, cm	28.0-88.0	44.0	14.7
Plant weight, g	28.0-269.8	95.2	63.9
Plant weight without pods, g	22.2-234.1	80.4	58.2
Pod length, cm	8.5-14.1	11.6	1.6
Number of pods per plant	5.0-20.0	9.6	4.1
Number of beans per pod	5.0-15.0	7.6	2.7
Weight of beans per plant, g	4-25.5	10.2	5.8
	Phaseolus vulgais		
Days to full flowering	30-45	43.5	4.8
Plant height, cm	28.0-92.0	52.8	19.4
Plant weight, g	16.0-42.7	24.6	8.6
Plant weight without pods, g	4.4-34.5	20.9	9.1
Pod length, cm	4.5-11.0	6.5	1.9
Number of pods per plant	2.0-7.0	3.8	1.7
Number of beans per pod	1.0-5.0	2.8	1.2
Weight of beans per plant, g	0.5-7.7	2.2	2.3

Chlorophyll values by a portable device showed that the range of chlorophyll units was higher between 0.21 and 1.92 in cowpea plants while the range in bean leaves was smaller between 0.15 and 1.37 (Figure 5). The chlorophyll content of bean leaves decreases from the beginning of vegetation - flowering till pod formation. For instance, the mean value for chlorophyll at the beginning of leaf petal measurement was 0.80, decreased to 0.52 during flowering and reached to 0,47 during to the pod formation. While in cowpea plants, an increase in the maximum values at the beginning of the vegetation from 1.38 to 1.92 during pod formation and a slight decrease in the minimum values from 0.44 to 0.19 during flowering to 0.15 in the last phase of cowpea development was observed.

Several studies have shown that cowpea is able to maintain high leaf water potential or high relative leaf water content during water stress by closing stomata and thus reducing dehydration (Souza et al., 2010). Studies based on chlorophyll fluorescence estimates in *Vigna unguiculata* the photosystem in these plants is quite resistant to water deficit. Only under extreme dry conditions inhibition of the photosynthetic apparatus can be reported. According to the authors, the reduction of CO_2 assimilation levels in water-stressed cowpea plants is largely dependent on stomata closure, which reduces available internal CO_2 and limits water loss by transpiration. This response appears to be effective in preventing a large reduction in leaf water potential and therefore appears to be the basis or avoiding dehydration in *V. unguiculata.*

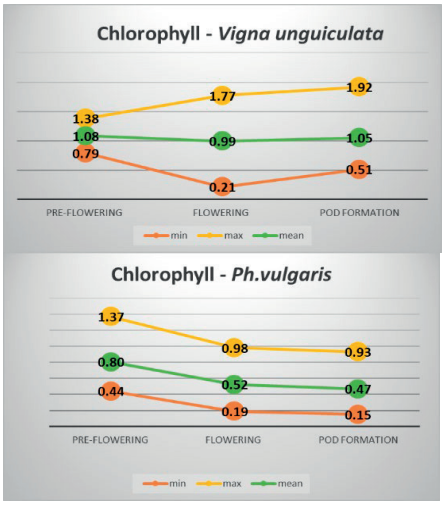


Figure 5. Trends of min., max. and mean Chlorophyll values in leaves of *V. unguiculata* and *Ph. vulgaris* during the three monitoring stages

For the both legumes, a decreasing trend in nitrogen content was observed during plant development. In the case of cowpea, a lesser decrease was recorded, with the mean values decreasing from 12.88 during pre flowering to

10.05 at the flowering period and reaching 9.16 at the pod formation. While in beans the decreasing trend is more from 11.39 to 5.89 to 3.94 - the decrease is 75% while in cowpea it is only 40%. The data presented on yield components found that there were relatively large differences in the measured components in different samples of the two legumes. (Table 1) Pearson's correlation coefficient was calculated to establish the relationship between the components measured by the portable devices and the yield components (Table 2).

Table 2. Pierson's coefficient between chlorophyll, NDVI and N contents and yield components of common bean *Ph. vulgaris* L. and cowpea (*V. unguiculata*)

	N	*NDVI*	*ChlM*
N	1.00		
NDVI	**0.69**	1.00	
ChlM	**0.75**	**0.62**	1.00
Days to full flowering	0.39	0.32	**0.57**
Plant height, cm	-0.02	0.01	-0.13
Plant weight, g	0.33	0.24	**0.52**
Plant weight without pods, g	0.34	0.23	**0.52**
Number of pods per plant	0.22	0.23	0.44
Weight of beans per plant (g)	0.23	0.23	0.42
Pod length (cm)	0.27	0.31	0.45
Number of beans per pod	0.28	0.28	**0.51**
Weight of beans per plant (g)	0.20	0.19	0.40

The highest correlation was found between nitrogen and chlorophyll content with R = 0.75, followed by nitrogen and NDVI with R = 0.69. A significant correlation was observed between chlorophyll and NDVI with R = 0.62.

As can be seen from the presented data on field components, there were relatively large differences in the measured indicators of the representatives of the two types of legumes. For example, plant height in cowpeas varied between 28.0 and 88.0 cm, while in beans the range was larger, between 28.0 and 92.0 cm. At optimal plant density, grain yield depends on the value of its structural components. The structural elements of yield, such as number of pods and seeds per plant, weight of pod and seeds per plant are indicators of type and varietal characteristics and as such are relatively constant values. A difference was found in the weight of the plant and the weight of the plant without pods, with data on the weight of the plant without pods varying between 22.2 and 234.1 g for cowpea and between 4.4 and 34.5 g for *Ph. vulgaris*. The number of pods per plant was between 5 and 20 for cowpea and between 2 and 7 for common bean. The aim of our study was to establish the relationship between morphological components obtained with spectral data of chlorophyll, NDVI and nitrogen values. In this regard, the large range between the values of the components of interest was obtained from different bean and cowpea samples, which is important for the higher precision of the study.

According to the obtained correlation coefficients, it was found that chlorophyll values a direct correlation with most of studied yield components. For example, a positive correlation was observed between chlorophyll content and days before mass flowering where R = 0.57. According to morphological parameters, such as plant weight and plant weight without pods the values of R = 0.52. For the reproductive organs, such as pods and beans, their number and weight, the correlation was found with the chlorophyll and the number of beans per pod with R = 0.51. Similar results were by reported Ramesh et al. (2002) for rice yield, the leaf chlorophyll content at 79 days after sowing correlated well with the grain yield of rice. Multiple regression models also indicated the dependence of rice yield on leaf chlorophyll content before and after flowering. The study of Islam et al. (2014) demonstrated also the importance of using SPAD mater for chlorophyll content in determining wheat yield under variable fertilizer management under field conditions.

CONCLUSIONS

The highest NDVI index in both legumes was recorded during the pre-flowering, after which there was a decline to the pod formation phase. The chlorophyll content in the leaves of the *Ph. vulgaris* accessions decreased from flowering to the pod formation, while the chlorophyll content in the leaves of cowpea (*V. unguiculata*) accessions was relatively stable with average values ranging from 1.08 to 1.05 from pre flowering to pod formation.

In both legumes, a decreasing trend in nitrogen content from the pre flowering to pod formation and maturation was observed, with a much

greater decrease of 75% in beans, while only 40% in cowpea. The results obtained for the relationship between the measured vegetative indices and yield components of the two legumes revealed that chlorophyll index was more significant compared to NDVI.

ACKNOWLEDGEMENTS

This work was supported by the Bulgarian Scientific Research Fund under Contract No. KP-06-H56/13 from 19 November 2021. The topic of the scientific research national project is: "Bioactive substances from legumes and medicinal species–features and potential for use in changing climatic conditions".

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

THE EVALUATION OF FERTILIZATION SCHEMES OPTIMISATION AT TWO ROW BARLEY CROP IN ORDER TO INCREASE THE HARVEST PROCESSING VALUE

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Abstract

As the cultivation of barley as a raw material for brewing beer is on an upward trend in Romania, to identifying an optimal fertilization scheme that enhances the quality of the grains is still a real challenge for producers and processors alike. In this context, the researches focused on the behavior of the Salamandre two row barley genotype in terms of grain yield achieved per unit area and the technical value of the harvest, against the background of the practice of differentiated fertilization schemes. The results of the research highlighted the positive impact of the administration of foliar fertilizers based on macro and microelements, in the phenophases considered critical for the supply of nutrients to plants. The highest values of the physical and chemical indicators that define the quality of grains in barley for brewing were obtained under the conditions of application of three foliar treatments with Poly Feed GG fertilizer applied in well-defined vegetation phases, against the background of administration at the time of establishment of the crop of 200 kg/ha of Complex fertilizers 20-20-0+13% Sulphur.

Key words*: two row barley, foliar fertilization, grain yield.*

INTRODUCTION

The management of different variables influences productivity, grain protein content and other quality parameters that have a direct impact on the quality of malt extracted from barley and barley grains for beer (Villiers et al., 1988).

The agronomic practices for increasing the quality of malt are quite different from those recommended by the technology of growing barley and barley for beer. Among them, the time and methods of sowing, the tillage system, irrigation, recommended nitrogen doses and the stage of nitrogen application greatly affect the productivity and quality of malt. Nitrogen is the main constituent of amino acids, which are precursors of proteins. Increasing nitrogen intake for a crop result in an increase in the protein content in the grains (Briggs, 1978; Briggs, 1998).

Sandhu (2006), reported that the grain yield of beer barley increased significantly with the application of a nitrogen dose of 78 kg/ha, as a result of plant height growth, efficient twinning, leaf area index value, dry matter accumulation (DMA), grain count/ear, mass of 1000 grains and nitrogen uptake, compared to applying lower doses of nitrogen.

Patel et al. (2004), reported an increase in grain production as a result of the application of nitrogen doses between 60-100 kg/ha. However, the increase in grain yield as a result of increasing the nitrogen dose administered to the barley crop to 100 kg/ha was insignificant compared to the 80 kg N/ha administration variant.

Gonzalez et al. (1992), in Toledo, Spain, observed that by increasing the nitrogen dose from 0 kg N/ha to 160 kg N/ha, biological production and grain production increased, as well as the Harvest Index.

It is possible that the application of a full dose of N in the early stages of plant growth may not be able to meet the nutritional needs of the barley crop for beer until maturity as the application of nitrogen in later stages can increase the protein content of the grains, thus decreasing the quality of the extracted malt (Malhi & Nyborg, 1992).

Roy & Singh (2006), reported that applying three doses of N at different times resulted in the highest protein and starch content of grains compared to applying nitrogen in a single dose.

Ruiter & Brooking (1994), showed that quality parameters could be improved by applying N post-anthesis without excessive accumulation of

N in grains, demonstrating that pre-anthesis nitrogen management ensured near-optimal barley crop growth for beer. Singh et al. (1974), reported that applying the full dose of N at the time of sowing keeps the nitrogen content and all other malt quality parameters within a desirable limit.
As a result, the present research aimed to test the influence of different foliar fertilization schemes on the behavior of the Salamandre barley variety and to identify the most optimal scheme so as to enhance the productive capacity of the variety under study, and the grains to meet the requirements imposed by the standards in force regarding the physical and chemical parameters that render the technological value of the grains intended for the beer industry.

MATERIALS AND METHODS

In order to determine the influence of differentiated foliar fertilization schemes on the productivity and technological value of barley grains intended for brewing in 2023 in Grădiștea, Giurgiu County, an experiment was designed in which the behavior of the Salamandre variety, grown at a sowing density of 350 germinable grains/m^2, was followed. Against the background of the basic fertilization of the soil in autumn by administering in autumn with the sowing of 200 kg/ha Complex 20-20-0+13% Sulphur.
During the vegetation period, in order to ensure the necessary nutrients for barley plants, foliar fertilizations were administered in the vegetation phases considered critical for the barley crop, namely: in the stem elongation phase, in the sprouting phase and in the grain formation and filling phase.
In order to establish the impact of the administration of foliar fertilizers on the orazoaica crop for beer, a bifactorial experiment was designed in subdivided plots, with three replications, the experimental factors tested in the research being the following:
Factor A - foliar fertilizers administered, with 3 graduations:
a1 - fertilized with MKP (0-52-34), 2% concentration;
a2 - fertilized with Poly Feed GG (9-10-38+3% MgO), 2% concentration;
a3 - fertilized with Multi K Mg (11-0-40+4% MgO), 2% concentration;
Factor B – the number of foliar treatments administered to the crop, with 3 graduations:
B1 - 1 foliar treatment (for straw elongation)
b2 - 2 foliar treatments (straw elongation and bellows phase)
b3 - 3 foliar treatments (for straw elongation, bellows and berry formation)
By combining the experimental factors tested in the research, 9 variants resulted, according to Table 1.

Table 1. Experimental variants tested in the two-row barley experiment

Experimental Variants		Factor B		
		b1	b2	b3
Factor A	a1	a1b1	a1b2	a1b3
	a2	a2b1	a2b2	a2b3
	a3	a3b1	a3b2	a3b3

The observations and determinations made during the research were aimed at both the evaluation of the grain yield of the Salamander variety, and the evaluation of the physical and chemical quality indicators required by the beer industry (physical purity of the grains, moisture of the grains, uniformity of the grains, germination capacity of the grains, protein and starch content of the grains.
The results obtained from the research were analyzed by the analysis of variance method for bifactorial experiments placed in subdivided plots, with three replicates.

RESULTS AND DISCUSSIONS

Against the background of practicing the foliar fertilization schemes tested in the research, the barley variety Salamandre achieved average grain yields of over 7000 kg/ha, regardless of the foliar fertilizer administered or the time of their administration, highlighting its productive capacity (Table 2).
The grain yield achieved in the 9 experimental variants was between 7317 kg/ha (V1), the lowest yield obtained under the conditions of applying during the vegetation period of the crop a single treatment in the stem elongation phase with the foliar fertilizer MKP (0-52-34) and 7870 kg/ha, in the experimental variant in which 3 foliar treatments were administered with Poly Feed GG (9-10-38+3% MgO), to elongation of the stem, to sprouting and to the formation and filling of grains (V6).

Table 2. Influence of foliar fertilization scheme on grains yield to Salamandre two row barley variety

Experimental variant	Physical yield (kg/ha)	Difference (kg/ha)	Significance degrees	Relative yield (%)	Difference (%)
V1 - 1 foliar fertilization with MKP (0-52-34)	7317	-308	ooo	95.96	-4.04
V2 - 2 foliar fertilizations with MKP (0-52-34)	7420	-205	ooo	97.31	-2.69
V3 - 3 foliar fertilizations with MKP (0-52-34)	7726	101	xxx	101.32	1.32
V4 - 1 foliar fertilization with Poly Feed GG (9-10-38+3% MgO)	7582	-43	o	99.44	-0.56
V5 - foliar fertilizations with Poly Feed GG (9-10-38+3% MgO)	7663	38	x	100.49	0.49
V6 - 3 foliar fertilizations with Poly Feed GG (9-10-38+3% MgO)	7870	245	xxx	103.21	3.00
V7 - 1 foliar fertilization with Multi K Mg (11-0-40+4% MgO)	7595	-30	o	99.60	-0.40
V8 - 2 foliar fertilizations with Multi K Mg (11-0-40+4% MgO)	7636	11	-	100.14	0.14
V9 - 3 foliar fertilizations with Multi K Mg (11-0-40+4% MgO)	7816	191	xxx	102.50	2,5
Experimental Average (Control)	7625	Control	Control	100.00	Control
	$DL_{5\%} = 23.775$; $DL_{1\%} = 77.611$; $DL_{0.1\%} = 91.921$				

Compared to the average of the experimental variants taken as a control, there were statistically very significantly negative (ooo) differences in the variants in which 2 foliar fertilizations with MKP were applied, significantly negative (o) in the variants in which a single foliar fertilization was administered in the elongation phase of the stem with Poly Feed GG or Multi K Mg (11-0-40+4% MgO), insignificant (-) as a result of the administration of two foliar fertilizations with Multi K Mg and very significantly positive (xxx), with production increases between 11 kg/ha and 191 kg/ha in the variants that benefited from three foliar treatments, regardless of the fertilizer used for this purpose. Following the determination of the physical purity of the barley grains harvested from the 9 experimental variants, it was observed that the values of this grain quality indicator exceeded in all tested variants the minimum value of 93% provided for this parameter by the national standard for brewing, with values between 96% and 98% being recorded, regardless of the fertilization scheme practiced (Table 3).

Table 3. Influence of foliar fertilization scheme on grains physical purity to Salamandre two row barley variety

Experimental Variant	Physical purity (%)	Difference (%)	Significance degrees
V1 - 1 foliar fertilization with MKP (0-52-34)	97	-0.11	-
V2 - 2 foliar fertilizations with MKP (0-52-34)	98	0.89	x
V3 - 3 foliar fertilizations with MKP (0-52-34)	98	0.89	x
V4 - 1 foliar fertilization with Poly Feed GG (9-10-38+3% MgO)	96	-1.11	o
V5 - foliar fertilizations with Poly Feed GG (9-10-38+3% MgO)	97	-0.11	-
V6 - 3 foliar fertilizations with Poly Feed GG (9-10-38+3% MgO)	98	0.89	x
V7 - 1 foliar fertilization with Multi K Mg (11-0-40+4% MgO)	96	-1.11	o
V8 - 2 foliar fertilizations with Multi K Mg (11-0-40+4% MgO)	97	-0.11	-
V9 - 3 foliar fertilizations with Multi K Mg (11-0-40+4% MgO)	97	-0.11	-
Experimental Average (Control)	97.11	Control	Control
	$DL_{5\%} = 0.874$; $DL_{1\%} = 1.446$; $DL_{0.1\%} = 2.706$		

The differences from the experimental control were quite small, ranging from 0.11 to 0.89 percentage points, with statistical assurance ranging from significantly negative (o) for variants V4 and V7, insignificant (-) for variants V1, V5, V8 and V9, to significantly positive (x) for variants V2, V3 and V6.

The determinations made after the seminal rest of the grains showed a moisture content that varied between 11.3% and 12.5% (Table 4), in all the 9 experimental variants tested in the research, this qualitative parameter of the grains being below the minimum limit of 14% required by the beer industry.

It is observed that the highest values of berry moisture were determined in the case of the experimental variants in which two foliar treatments were administered (in the elongation phase of the spike and in the phase of berry formation and filling) with Poly Feed GG and Multi K Mg, with very significantly positive differences (xxx) compared to the average of the experimental variants (Control).

Table 4. Influence of foliar fertilization scheme on grains moisture content to Salamandre two row barley variety

Experimental Variant	Moisture content (%)	Difference (%)	Significance degrees
V1 - 1 foliar fertilization with MKP (0-52-34)	11.3	-0.76	ooo
V2 - 2 foliar fertilizations with MKP (0-52-34)	11.7	-0.36	ooo
V3 - 3 foliar fertilizations with MKP (0-52-34)	11.9	-0.16	o
V4 - 1 foliar fertilization with Poly Feed GG (9-10-38+3% MgO)	11.9	-0.16	o
V5 - foliar fertilizations with Poly Feed GG (9-10-38+3% MgO)	12.3	0.24	xxx
V6 - 3 foliar fertilizations with Poly Feed GG (9-10-38+3% MgO)	12.4	0.34	xxx
V7 - 1 foliar fertilization with Multi K Mg (11-0-40+4% MgO)	12.3	0.24	xxx
V8 - 2 foliar fertilizations with Multi K Mg (11-0-40+4% MgO)	12.3	0.24	xxx
V9 - 3 foliar fertilizations with Multi K Mg (11-0-40+4% MgO)	12.5	0.44	xxx
Experimental Average (Control)	12.06	Control	Control
	$DL_{5\%} = 0.091$; $DL_{1\%} = 0.127$; $DL_{0.1\%} = 0.180$		

Following the determinations regarding the weight of the grains, it is noted that the Salamandre barley variety reacted very well to the administration of foliar fertilizers during the vegetation period, from the 9 experimental variants tested during the research, grains with a weight that exceeded the minimum values imposed by the national standard for the beer industry were harvested, respectively 65 kg/hl for the volumetric weight and 45 g for the mass of 1000 grains.

Thus, the mass values of 1000 grains varied between 46.7 g and 50.6 g, the highest values of this grain quality indicator being obtained in the experimental variants in which 3 foliar fertilizations with MKP (50.6 g) and Poly Feed GG (50.3 g) were applied, respectively the V3 and V6 variants (Table 5).

The foliar fertilizers tested recorded very statistically significant positive (xxx) differences from the average experience under the administration of two or three foliar treatments with MKP, Poly Feed GG and Multi K Mg (V2, V3, V5, V6 and V9), and from significantly negative (o) to very significantly negative (ooo) by administering a single foliar fertilization in the elongation phase of the stems (V1, V4, V7 and V8).

Table 5. Influence of foliar fertilization scheme on grains weight to Salamandre two row barley variety

Experimental Variant	Weight of 1000 grains (g)	Diff. (g)	Signif. degrees	Volumetric weight (kg/hl)	Diff. (kg/hl)	Signif. degrees
V1 - 1 foliar fertilization with MKP (0-52-34)	48.3	-0.5	ooo	66.2	-0.4	o
V2 - 2 foliar fertilizations with MKP (0-52-34)	49.9	1.1	xxx	66.8	0.2	o
V3 - 3 foliar fertilizations with MKP (0-52-34)	50.6	1.8	xxx	67.9	1.3	xxx
V4 - 1 foliar fertilization with Poly Feed GG (9-10-38+3% MgO)	47.4	-1.4	o	66.1	-0.5	o
V5 - foliar fertilizations with Poly Feed GG (9-10-38+3% MgO)	49.6	0.8	xxx	66.4	-0.2	o
V6 - 3 foliar fertilizations with Poly Feed GG (9-10-38+3% MgO)	50.3	1.5	xxx	67.2	0.6	xx
V7 - 1 foliar fertilization with Multi K Mg (11-0-40+4% MgO)	46.7	-2.2	oo	65.8	-0.8	oo
V8 - 2 foliar fertilizations with Multi K Mg (11-0-40+4% MgO)	47.3	-1.5	o	66.1	-0.5	o
V9 - 3 foliar fertilizations with Multi K Mg (11-0-40+4% MgO)	49.1	0.3	xxx	66.9	0.3	x
Experimental Average (Control)	48.8	Control	Control	66.6	Control	Control
	$DL_{5\%} = 0.119$; $DL_{1\%} = 0.167$; $DL_{0.1\%} = 0.235$			$DL_{5\%} = 0.12$; $DL_{1\%} = 0.58$; $DL_{0.1\%} = 0.86$		

The volumetric weight values of the grains harvested from the 9 experimental variants were between 65.8 kg/hl and 67.9 kg/hl (Table 5), with very significantly positive (xxx) differences compared to the average of the experimental variants (control), against the background of the administration of three foliar fertilizations with MKP (V3), distinctly significantly positive (xx) by applying three treatments with Poly Feed GG (V6) and significantly positive (x) following the administration of the same number of foliar treatments with Multi K Mg (V9).

Table 6. Influence of foliar fertilization scheme on grains uniformity to Salamandre two row barley variety

Experimental Variant	Class I 2.8 mm (%)	Diff. (%)	Signif. degrees	Class II 2.5 mm (%)	Diff. (%)	Signif. degrees
V1 - 1 foliar fertilization with MKP (0-52-34)	89	-4.22	ooo	11	4.23	xxx
V2 - 2 foliar fertilizations with MKP (0-52-34)	91	-2.22	oo	9	2.23	x
V3 - 3 foliar fertilizations with MKP (0-52-34)	93	-0.22	-	7	0.23	-
V4 - 1 foliar fertilization with Poly Feed GG (9-10-38+3% MgO)	93	-0.22	-	7	0.23	-
V5 - foliar fertilizations with Poly Feed GG (9-10-38+3% MgO)	95	1.78	xx	5	-1.77	o
V6 - 3 foliar fertilizations with Poly Feed GG (9-10-38+3% MgO)	97	3.78	xxx	3	-3.77	ooo
V7 - 1 foliar fertilization with Multi K Mg (11-0-40+4% MgO)	91	-2.22	oo	9	2.23	x
V8 - 2 foliar fertilizations with Multi K Mg (11-0-40+4% MgO)	94	0.78	-	6	-0.77	-
V9 - 3 foliar fertilizations with Multi K Mg (11-0-40+4% MgO)	96	2.78	xxx	4	-2.77	oo
Experimental Average (Control)	93.22	Control	Control	6.77	Control	Control
	$DL_{5\%} = 1.187$; $DL_{1\%} = 1.666$; $DL_{0.1\%} = 2.352$			$DL_{5\%} = 1.678$; $DL_{1\%} = 2.356$; $DL_{0.1\%} = 3.326$		

Against the background of practicing the fertilization schemes tested in the experience with barley for beer, the Salamandre variety has achieved grains with a high degree of uniformity, in all 9 experimental variants the grains resulting from the harvest of the crop falling into the first quality class in terms of grain uniformity, in accordance with both the national standard (minimum 80% grains with a diameter greater than 2.5 mm), and with the European standard (minimum 85% grains with a diameter greater than 2.5 mm) for barley intended for brewing.

The largest and most uniform grains were harvested in the experimental variants in which 2 treatments were administered during the vegetation period of the crops with Poly Feed GG (V5 and V6) and Multi K Mg (V8 and V9), the degree of uniformity of the grains exceeding 94% in these experimental variants (Table 6), with distinctly significantly positive (xx) and very significantly positive (xxx) differences compared to the average of the experimental variants (control).

By applying the foliar fertilizer MKP, the differences from the average experience were very significantly negative (oo) as a result of the administration of a single foliar treatment, distinctly significantly negative (oo), by applying two foliar fertilizations and insignificant (-) by applying three foliar treatments (Table 6).

The germination capacity of the grains did not register significant differences between the 9 experimental variants taken in the study, the values of this barley grain quality parameter required by the beer industry varying between 95% and 98%, the grains harvested from the 9 experimental variants exceeding, after 72 hours from the moment of their placement on the germination layer, the minimum germination capacity scale of 92% provided by the standard for this destination (Table 7).

Table 7. Influence of foliar fertilization scheme on grains germination capacity to Salamandre two row barley variety

Experimental Variant	Germination capacity (%)	Difference (%)	Significance degrees
V1 - 1 foliar fertilization with MKP (0-52-34)	95	-2.0	oo
V2 - 2 foliar fertilizations with MKP (0-52-34)	96	-1.0	o
V3 - 3 foliar fertilizations with MKP (0-52-34)	98	1.0	x
V4 - 1 foliar fertilization with Poly Feed GG (9-10-38+3% MgO)	97	0.0	-
V5 - foliar fertilizations with Poly Feed GG (9-10-38+3% MgO)	98	1.0	x
V6 - 3 foliar fertilizations with Poly Feed GG (9-10-38+3% MgO)	98	1.0	x
V7 - 1 foliar fertilization with Multi K Mg (11-0-40+4% MgO)	96	-1.0	o
V8 - 2 foliar fertilizations with Multi K Mg (11-0-40+4% MgO)	97	0.0	-
V9 - 3 foliar fertilizations with Multi K Mg (11-0-40+4% MgO)	98	1.0	x
Experimental Average (Control)	97	Control	Control
	$DL_{5\%} = 0.874$; $DL_{1\%} = 1.446$; $DL_{0.1\%} = 2.706$		

Compared to the average experience (control), in the case of this physiological parameter of barley grain quality, statistically assured differences were recorded from distinctly

significant negative (oo) by applying a single foliar treatment with MKP (V1), to significantly positive (x), as a result of the application of two or three foliar fertilizations, regardless of the fertilizer used for this purpose (V3, V5, V6 and V9).

Table 8. Influence of foliar fertilization scheme on grains protein content to Salamandre two row barley variety

Experimental variant	Protein content (%)	Difference (%)	Significance degrees
V1 - 1 foliar fertilization with MKP (0-52-34)	9.6	-0.53	ooo
V2 - 2 foliar fertilizations with MKP (0-52-34)	9.6	-0.53	ooo
V3 - 3 foliar fertilizations with MKP (0-52-34)	9.8	-0.33	oo
V4 - 1 foliar fertilization with Poly Feed GG (9-10-38+3% MgO)	9.8	-0.33	oo
V5 - foliar fertilizations with Poly Feed GG (9-10-38+3% MgO)	9.8	-0.33	oo
V6 - 3 foliar fertilizations with Poly Feed GG (9-10-38+3% MgO)	10.1	-0.03	-
V7 - 1 foliar fertilization with Multi K Mg (11-0-40+4% MgO)	10.6	0.47	xxx
V8 - 2 foliar fertilizations with Multi K Mg (11-0-40+4% MgO)	10.8	0.67	xxx
V9 - 3 foliar fertilizations with Multi K Mg (11-0-40+4% MgO)	11.1	0.97	xxx
Experimental Average (Control)	10.13	Control	Control
	$DL_{5\%} = 0.162$; $DL_{1\%} = 0.237$; $DL_{0.1\%} = 0.364$		

Analyzing the results obtained from the determination of the protein content of the grains, results that are centralized in Table 8, it is observed that, against the background of the practice of differentiated foliar fertilization schemes, the Salamandre variety performed very well in terms of this quality parameter, with values between 9.6% and 11.1% protein content of the grains harvested from the 9 experimental variants falling within the limits of 9.5-11.5% provided by the standard for Intended for brewing. Making a comparative analysis between the experimental variants tested during the research, the variants that were administered foliar fertilitization with MKP and Poly Feed GG stand out in terms of grain protein content, the grain proteins having values ranging between 9.6% and 9.8%, regardless of the number of foliar fertilizations applied or the time of their treatment.

With a starch content between 61% and 62.9% (Table 9), the Salamandre variety exceeded the minimum scale of 60% provided by the national standard for barley for the beer industry, the fertilization schemes practiced in the research having a direct and significant influence on this quality indicator. By administering the foliar fertilizer Multi K Mg, the starch content values recorded the highest values, exceeding 61.8%, regardless of the number of foliar treatments applied during the vegetation period of the barley crop.

The differences from the average of the experimental variants were statistically ensured from very significant negative (ooo) by administering a single foliar treatment in the elongation phase of the stem with MKP, to very significantly positive (xxx) following the administration of three foliar treatments (in the stem elongation phase, in the sprouting phase and in the bean formation phase) with Poly Feed GG and Multi K Mg).

Table 9. Influence of foliar fertilization scheme on grains starch content to Salamandre two row barley variety

Experimental variant	Starch content (%)	Difference (%)	Significance degrees
V1 - 1 foliar fertilization with MKP (0-52-34)	61.0	-0.97	ooo
V2 - 2 foliar fertilizations with MKP (0-52-34)	61.4	-0.57	oo
V3 - 3 foliar fertilizations with MKP (0-52-34)	61.8	-0.17	o
V4 - 1 foliar fertilization with Poly Feed GG (9-10-38+3% MgO)	61.7	-0.27	oo
V5 - foliar fertilizations with Poly Feed GG (9-10-38+3% MgO)	61.9	-0.07	-
V6 - 3 foliar fertilizations with Poly Feed GG (9-10-38+3% MgO)	62.9	0.93	xxx
V7 - 1 foliar fertilization with Multi K Mg (11-0-40+4% MgO)	61.8	-0.17	o
V8 - 2 foliar fertilizations with Multi K Mg (11-0-40+4% MgO)	62.4	0.43	xx
V9 - 3 foliar fertilizations with Multi K Mg (11-0-40+4% MgO)	62.9	0.93	xxx
Experimental Average (Control)	61.97	Control	Control
	$DL_{5\%} = 0.135$; $DL_{1\%} = 0.221$; $DL_{0.1\%} = 0.582$		

CONCLUSIONS

The results obtained for the Salamandre barley variety grown in 2024 under differentiated fertilization conditions, highlighted the following:

The highest grain production (7870 kg/ha) was obtained in an experimental version in which three foliar treatments with Poly Feed GG (9-10-38+3% MgO) were administered, respectively, in the stem elongation phase, in the sprouting phase and in the berry formation phase.

Regardless of the foliar fertilizer administered and the number of treatments carried out during the vegetation period of the barley crop, the Salamandre variety obtained physical purity values above the minimum limit of 93% provided by the national standard for barley grains intended for brewing.

In terms of the moisture content of the grains after their seminal rest, values below the maximum limit of 14% required by the brewing destination were obtained, regardless of the fertilization scheme practiced during the vegetation period of the barley crop.

The Salamandre barley variety reacted very well to the administration of foliar fertilizers during the crop's vegetation period, exceeding the minimum values of 65 kg/hl for volumetric weight and 45 g for the mass of 1000 grains provided by the national standard for the beer industry, the highest values of these parameters being obtained as a result of the administration of three foliar treatments with MKP and Poly Feed GG.

At the Salamandre barley variety, grains with a high degree of uniformity were obtained, classified in the first quality class, both according to the national standard and according to the European standard for barley, as a raw material in the chain - Brewing.

With a germination capacity between 95% and 98%, the barley grains harvested from the 9 experimental variants exceeded the minimum scale of 92% provided by the national standard for the beer industry, regardless of the fertilization scheme practiced during the crop's vegetation period.

Salamandre variety performed very well in terms of grains protein content, falling within the limits of 9.5-11.5% provided by the standard for Intended for brewing, with values between 9.6% and 11.1% protein the variants that were administered foliar fertilitization with MKP and Poly Feed GG stand out in terms of this parameter , the grain proteins having values ranging between 9.6% and 9.8% regardless of the fertilization scheme practiced.

For the Salamandre barley variety, against the background of the practice of differentiated schemes, values of the starch content of the grains were recorded that exceeded 60%, a scale provided by the national standard for barley intended for the beer industry.

Among the fertilization schemes tested during the research, the optimal fertilization scheme proved to be the one in which three foliar treatments were administered, in the vegetation phases considered critical for nutrients, with the Poly Feed GG fertilizer, the Salamandre variety achieving clearly superior grain productions and with high technological value, in accordance with the requirements provided in the quality standards for the beer industry.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

CRITICAL SALINITY THRESHOLDS IMPACTING WHEAT GERMINATION: DETERMINING DOSE-DEPENDENT RESPONSES ACROSS VARIETIES

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Abstract

*The study aimed to establish the critical salinity dose that can be tolerated by 16 wheat varieties from Romania under controlled conditions. The tested varieties were Alex, Andrada, Bezostaia, Boema, Arieșan, Ciprian, Esențial, Faur, Glosa, Granny, Fundulea, Miranda, Otilia, Pădureni, Taisa and Transilvania. Critical salinity levels (mM NaCl) were tested respectively D1-100, D2-125, D3-150; D4-175; D5-200; D6-225; D7-250; and control (C) with distilled water. The assessment was made according to first and second principal growth stage on BBCH scale (**B**iologische Bundesanstalt, **B**undessortenamt und **CH**emische Industrie) and the following parameters were calculated: germination percentage (GP), germination index (GI), total germinated seeds (TGS) and relative salt injury rate (RSIR). After 7 days, at 250 mM NaCl, the wheat varieties reached overall the maximum developmental stage of BBCH 07. Lower RSIR was obtained by Andrada variety a result of an increased salinity resistance, while Taisa, Transilvania, and Pădureni proved lower resistance to stress, marked by increased RSIR coefficient. Germination standard parameters highlighted the critical salinity thresholds between 225-250 mM NaCl with different resistance threshold between varieties.*

***Key words**: abiotic stress, early growth stages, relative salt injury rate, wheat tolerance.*

INTRODUCTION

Soil salinity is an abiotic stress affecting a significant proportion of agricultural cropland worldwide (Nehra et al., 2024). While the Orient is facing already the harmful effects of salinity (Zaman et al., 2018) and researchers are looking for strategies to adapt and improve plants in the presence of this stressor (Fita et al., 2015), the European continent is facing an expansion of salinization (Daliakopoulos et al., 2016). These worrying aspects sets considerable focus on soil health (Mustafa et al., 2019). Soil health is a concept of real interest (Panagos et al., 2024), as it is an essential and fundamental element in achieving the Sustainable Development Goals (SDGs) of the Sustainable Development Agenda for 2030 adopted by the United Nations (Head et al., 2020). Achieving and maintaining soil health is of high interest, particularly for the second Sustainable Development Goal (SDG 2) of ending global hunger (Lal et al., 2021). By focusing on soil health, the healthy lives and well-being of the population (SDG 3) can be enhanced (Howden-Chapman et al., 2017). Additionally, soil health is closely linked to the sixth SDG which aims to ensure clean water sources. (Bonfante et al., 2020) Indirectly, maintaining soil quality supports SDG 12, which targets responsible consumption and production, as well as SDGs 13 and 15, which focus on climate action and the conservation of terrestrial ecosystems (Mikhailova et al., 2023). Achieving soil health ultimately contributes to SDG 8 and SDG 1 by promoting productive agriculture and generating economic opportunities through employment (Visser et al., 2019).

Salinity effects, on both soil health and as a stress for plants, pose a significant threat to agricultural yields and global food security. In plant organisms, salinity effect is first felt by the osmotic stress onset caused by the altered osmotic potential of soil solution (Soni et al., 2023). This abiotic stress affects all the plants phenological stages (Okon, 2019), but the most sensitive of these are germination and anthesis (El Sabagh et al., 2021), while leaves senescence can set in prematurely (Allu et al.,

2014). At the same time, salt stress induces an ionic imbalance (Hualpa-Ramirez et al., 2024), affects photosynthesis (Mahavar et al., 2024), and leads to a reduction in biomass accumulation (Huanhe et al., 2024). All these aspects are reflected in low crop productivity and production, which in the context of climate change and growing population is a real threat to the global food security (van den Burg et al., 2024). The energy source from the daily diet is based on carbohydrate intake (Jequier, 1994). The main sources of carbohydrates and fibers are cereals (McKevith, 2004), among which wheat *Triticum aestivum* L., through its by-products, is a favourite among cultivated species (Kumar et al., 2011). Global wheat production recorded in 2022/2023 exceeded 800 million tons, but forecasts for 2023/2024 and 2024/2025 predict declines (FAO, 2024). Wheat yield varies slightly from year to year depending on ecophysiological conditions (Huzsvai et al., 2022). Under salinity conditions, wheat plants have diverse responses depending on the salinity concentration and the stress exposure time (Negrão et al., 2017). Wheat seed germination is delayed and shoot growth and development are affected by the stressor (Akbarimoghaddam et al., 2011). Germination is a phenological stage highly sensitive to ecophysiological conditions. Saline stress, depending on the concentration, can inhibit the process and even delay it by the onset of water stress and ion toxicity (El Sabagh et al., 2021). Plant height also decreases when wheat is subjected to successive saline treatments (Kandil et al., 2012). Biomass accumulation is also influenced by the low photosynthetic rate caused by prolonged exposure to stress (Paul et al., 2019). Both underground and aboveground organs are affected by a decrease in root biomass and leaf biomass, which ultimately influence crop production (Chourasia et al., 2022). Considering all these aspects, the study of wheat physiological processes in response to salinity is of real interest.

The study aimed to establish the maximum salinity dose that can be tolerated by 16 wheat varieties during germination and early BBCH stages under controlled conditions. The objectives were to conduct daily monitoring of the germination process, calculate the germination percentage and index, assess the impact of salinity stress following relative salt injury rate based on responses of 16 wheat varieties to eight increasing doses.

MATERIALS AND METHODS

The experiment was set up on February 5th, 2024, in the Plant Physiology Department research laboratory of USAMV Cluj-Napoca. The plant material was represented by a collection of 16 wheat *Triticum aestivum* L. varieties from the Agricultural Research and Development Station (ARDS) Turda. The 16 varieties were Alex, Andrada, Bezostaia, Boema, Arieșan, Ciprian, Esențial, Faur, Glosa, Granny, Fundulea, Miranda, Otilia, Pădureni, Taisa and Transilvania. For each variety, 20 seeds per replication were tested in 4 replicates. Each variety was subjected to a salt stress gradient, with seven salinity doses (D_1 - 100 mM NaCl, D_2 - 125 mM NaCl, D_3 - 150 mM NaCl; D_4 - 175 mM NaCl; D_5 - 200 mM NaCl; D_6 - 225 mM NaCl; D_7 - 250 mM NaCl; the results were compared to a control (C) with water.

Experimental conditions were daily monitored, both humidity, temperature, and photoperiod. The average humidity and temperature recorded for the entire experiment were 31.79±0.71% and 23.50±0.19°C. The photoperiod consisted of 11 hours of daylight and 13 hours of darkness. Setting up the experiment involved preparing the seven saline solutions using a high-precision balance. The germination process was carried out in transparent polystyrene Petri dishes (Alom et al., 2016) with a diameter of 9 cm. On the bottom of these plates were placed three 70% cotton disks of 6 cm diameter. The three pads were soaked in 25 ml of water or solution corresponding to the saline dose applied. Subsequently, the 20 seeds of each variant were applied on the wet layer. A volume of 3.5 mL of the dose corresponding to each variant was applied every two days. The germination process was monitored during the experimental period.

Germination percentage (GP), germination index (GI), total germinated seeds (TGS) and relative salt injury rate (RSIR) (Yohannes et al., 2020) were determined in the defining days, i.e.

on the fourth when the seeds reached mean germination time (Duda et al., 2003) and seventh day when the process ended (Woku et al., 2016). These parameters were calculated using the formulas:

$$GP = \frac{NUMBER\ OF\ GERMINATED\ SEEDS}{TOTAL\ NUMBER\ OF\ TESTED\ SEEDS} \times 100$$

$$GI = \frac{DAY\ AFTER\ SOWING \times NUMBER\ OF\ GERMINATED\ SEEDS}{TOTAL\ NUMBER\ OF\ TESTED\ SEEDS}$$

$$RSIR = \frac{GP(control) - GP(saline\ dose)}{GP(control)} \times 100$$

Relative salt injury rate (RSIR) is a parameter that can give details on how salt stress intervenes in the germination process, relating each variant to the performance recorded in the control. All data were recorded in synthetic tables, where the red or dark blue color in each cell indicates the direction of treatment effects. The negative value (marked in red) of the RSIR is associated with a positive effect induced by the application of the dose. In contrast, high positive values (marked in dark blue) indicate a strong negative impact of the doses on the varieties.

The germination percentage (GP) (Kandil et al., 2012) is directly influenced by and related to the total germinated seeds (TGS), while the germination index (GI) (Yohannes et al., 2020) depends on the assessment day and also on the TGS. In this way, the dependence relationship between the parameters is evident through the distribution of the recorded values and mirrors a quantitative analysis of the germination process. All the values were then introduced into a database. Mean values and standard errors were obtained after performing the analyse of variance test using the statistical LSD test analysis with the software RStudio (R Core Team, 2020), based on "psych" (Corcoz et al., 2022; Revelle, 2017; Stoian et al., 2022) and "agricolae" (de Mendiburu, 2019; Stoian et al., 2016) packages. Mean values for all the parameters were then processed using conditional formatting for highlighting lower-higher values. The best performances were marked with the dark red, while the lower values with dark blue. The wheat seed germination and leaf development noted on BBCH growth scale (BBCH Working Group, 2001).

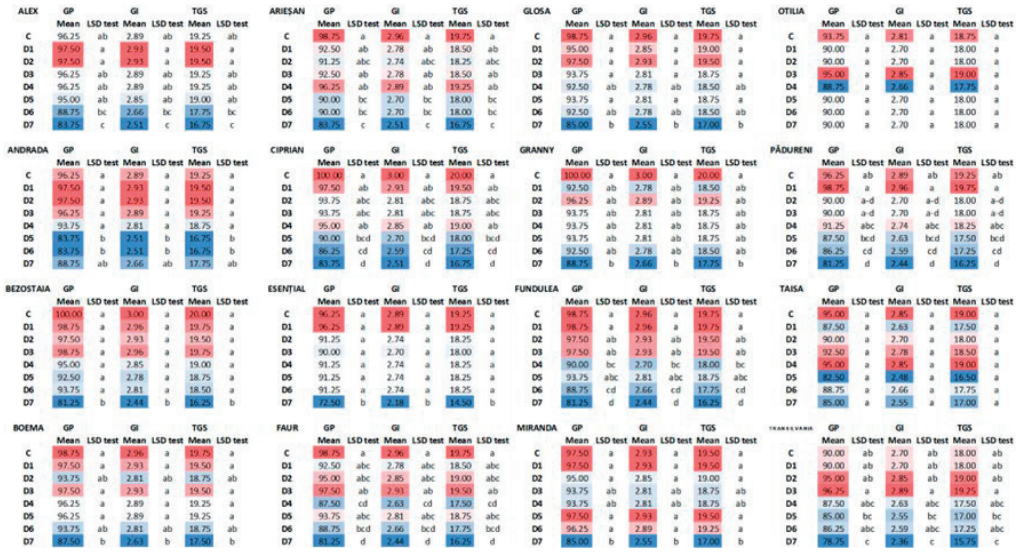
RESULTS AND DISCUSSIONS

Germination parameters-GP, GI and TGS

The GP, GI and TGS gradually decreased with the seven saline stress doses application. However, this aspect outlined in most of the varieties shows some exceptions on the fourth day of germination. These atypical aspects were outlined in the Miranda variety, which reached maximum of these three parameters at C (control), D1 (100 mM NaCl) and even at D5 (200 mM NaCl) (Figure 1). Similarly, Taisa showed same trends at C and D4 (175 mM NaCl), Otilia and Transilvania at D3 (150 mM NaCl) and Pădureni at D2 (125 mM NaCl) and D3. The two doses, D1 and D2, also stimulated the germination of the wheat varieties Andrada and Alex. However, the presence of salt stress did not significantly affect the germination process of Otilia and Taisa. Another interesting aspect is outlined for the varieties Bezostaia, Boema, Esențial, Glosa, Granny and Miranda, where the three parameters were significantly reduced only when exposed to the 250 mM NaCl dose. On the other hand, all the other varieties exhibited a significantly lower performance with the gradual increase of the salt doses. Overall, all cultivars reached at least the GP 95% saline treatment dose. A share of 37.5% from the 16 varieties, Arieșan, Ciprian, Miranda, Otilia, Taisa and Transilvania showed the most uneven GP distributions among the seven treatment doses. It is possible that this aspect outlines the tolerance of each variety to a certain key dose which has a stimulating effect on the process. The low tolerance of Pădureni and Esențial wheat observed in the fourth day is indicated by the fact that they only reach a GP higher than 95% at C and D1. Moreover, the application of higher doses over D4 gradually decrease the parameters analyzed in the case of Pădureni. A lower share of 12.5% from overall varieties, Glosa and Granny, reached a germination percentage higher than 95% only up to D2.

The degree of tolerance, as indicated by the parameter values, increased at D3 for Andrada and Fundulea, and at D4 for Bezostaia and Faur. The most tolerant varieties, after analyzing the values, were Alex and Boema, representing the wheat seeds that registered GP higher than 95% up to the fifth dose of salt

stress. The germination advancement on the fourth day is influenced not only by the salinity stress applied (Maas and Poss, 1989) but also by the genetic tolerance inherent (Amro et al., 2022) to each wheat variety.



ALEX	GP		GI		TGS	
	Mean	LSD test	Mean	LSD test	Mean	LSD test
C	96.25	ab	2.89	ab	19.25	ab
D1	97.50	a	2.93	a	19.50	a
D2	97.50	a	2.93	a	19.50	a
D3	96.25	ab	2.89	ab	19.25	ab
D4	96.25	ab	2.89	ab	19.25	ab
D5	95.00	ab	2.85	ab	19.00	ab
D6	88.75	bc	2.66	bc	17.75	bc
D7	83.75	c	2.51	c	16.75	c

ARIEȘAN	GP		GI		TGS	
	Mean	LSD test	Mean	LSD test	Mean	LSD test
C	98.75	a	2.96	a	19.75	a
D1	92.50	ab	2.78	ab	18.50	ab
D2	91.25	abc	2.74	abc	18.25	abc
D3	92.50	ab	2.78	ab	18.50	ab
D4	96.25	ab	2.89	ab	19.25	ab
D5	90.00	bc	2.70	bc	18.00	bc
D6	90.00	bc	2.70	bc	18.00	bc
D7	83.75	c	2.51	c	16.75	c

GLOSA	GP		GI		TGS	
	Mean	LSD test	Mean	LSD test	Mean	LSD test
C	98.75	a	2.96	a	19.75	a
D1	95.00	a	2.85	a	19.00	a
D2	97.50	a	2.93	a	19.50	a
D3	93.75	a	2.81	a	18.75	a
D4	92.50	ab	2.78	ab	18.50	ab
D5	93.75	a	2.81	a	18.75	a
D6	92.50	ab	2.78	ab	18.50	ab
D7	85.00	b	2.55	b	17.00	b

OTILIA	GP		GI		TGS	
	Mean	LSD test	Mean	LSD test	Mean	LSD test
C	93.75	a	2.81	a	18.75	a
D1	90.00	a	2.70	a	18.00	a
D2	90.00	a	2.70	a	18.00	a
D3	95.00	a	2.85	a	19.00	a
D4	88.75	a	2.66	a	17.75	a
D5	90.00	a	2.70	a	18.00	a
D6	90.00	a	2.70	a	18.00	a
D7	90.00	a	2.70	a	18.00	a

ANDRADA	GP		GI		TGS	
	Mean	LSD test	Mean	LSD test	Mean	LSD test
C	96.25	a	2.89	a	19.25	a
D1	97.50	a	2.93	a	19.50	a
D2	97.50	a	2.93	a	19.50	a
D3	96.25	a	2.89	a	19.25	a
D4	93.75	a	2.81	a	18.75	a
D5	83.75	b	2.51	b	16.75	b
D6	83.75	b	2.51	b	16.75	b
D7	88.75	ab	2.66	ab	17.75	ab

CIPRIAN	GP		GI		TGS	
	Mean	LSD test	Mean	LSD test	Mean	LSD test
C	100.00	a	3.00	a	20.00	a
D1	97.50	ab	2.93	ab	19.50	ab
D2	93.75	abc	2.81	abc	18.75	abc
D3	93.75	abc	2.81	abc	18.75	abc
D4	95.00	ab	2.85	ab	19.00	ab
D5	90.00	bcd	2.70	bcd	18.00	bcd
D6	86.25	cd	2.59	cd	17.25	cd
D7	83.75	d	2.51	d	16.75	d

GRANNY	GP		GI		TGS	
	Mean	LSD test	Mean	LSD test	Mean	LSD test
C	100.00	a	3.00	a	20.00	a
D1	92.50	ab	2.78	ab	18.50	ab
D2	96.25	ab	2.89	ab	19.25	ab
D3	93.75	ab	2.81	ab	18.75	ab
D4	93.75	ab	2.81	ab	18.75	ab
D5	93.75	ab	2.81	ab	18.75	ab
D6	92.50	ab	2.78	ab	18.50	ab
D7	88.75	b	2.66	b	17.75	b

PĂDURENI	GP		GI		TGS	
	Mean	LSD test	Mean	LSD test	Mean	LSD test
C	96.25	ab	2.89	ab	19.25	ab
D1	98.75	a	2.96	a	19.75	a
D2	90.00	a-d	2.70	a-d	18.00	a-d
D3	90.00	a-d	2.70	a-d	18.00	a-d
D4	91.25	abc	2.74	abc	18.25	abc
D5	87.50	bcd	2.63	bcd	17.50	bcd
D6	86.25	cd	2.59	cd	17.25	cd
D7	81.25	d	2.44	d	16.25	d

BEZOSTAIA	GP		GI		TGS	
	Mean	LSD test	Mean	LSD test	Mean	LSD test
C	100.00	a	3.00	a	20.00	a
D1	98.75	a	2.96	a	19.75	a
D2	97.50	a	2.93	a	19.50	a
D3	98.75	a	2.96	a	19.75	a
D4	95.00	a	2.85	a	19.00	a
D5	92.50	a	2.78	a	18.75	a
D6	93.75	a	2.81	a	18.50	a
D7	81.25	b	2.44	b	16.25	b

ESENȚIAL	GP		GI		TGS	
	Mean	LSD test	Mean	LSD test	Mean	LSD test
C	96.25	a	2.89	a	19.25	a
D1	96.25	a	2.89	a	19.25	a
D2	91.25	a	2.74	a	18.25	a
D3	90.00	a	2.70	a	18.00	a
D4	91.25	a	2.74	a	18.25	a
D5	91.25	a	2.74	a	18.25	a
D6	91.25	a	2.74	a	18.25	a
D7	72.50	b	2.18	b	14.50	b

FUNDULEA	GP		GI		TGS	
	Mean	LSD test	Mean	LSD test	Mean	LSD test
C	98.75	a	2.96	a	19.75	a
D1	98.75	a	2.96	a	19.75	a
D2	97.50	ab	2.93	ab	19.50	ab
D3	97.50	ab	2.93	ab	19.50	ab
D4	90.00	bc	2.70	bc	18.00	bc
D5	93.75	abc	2.81	abc	18.75	abc
D6	88.75	cd	2.66	cd	17.75	cd
D7	81.25	d	2.44	d	16.25	d

TAISA	GP		GI		TGS	
	Mean	LSD test	Mean	LSD test	Mean	LSD test
C	95.00	a	2.85	a	19.00	a
D1	87.50	a	2.63	a	17.50	a
D2	90.00	a	2.70	a	18.00	a
D3	92.50	a	2.78	a	18.50	a
D4	95.00	a	2.85	a	19.00	a
D5	82.50	a	2.48	a	16.50	a
D6	88.75	a	2.66	a	17.75	a
D7	85.00	a	2.55	a	17.00	a

BOEMA	GP		GI		TGS	
	Mean	LSD test	Mean	LSD test	Mean	LSD test
C	98.75	a	2.96	a	19.75	a
D1	97.50	a	2.93	a	19.50	a
D2	93.75	ab	2.81	ab	18.75	ab
D3	97.50	a	2.93	a	19.50	a
D4	96.25	a	2.89	a	19.25	a
D5	96.25	a	2.89	a	19.25	a
D6	93.75	ab	2.81	ab	18.75	ab
D7	87.50	b	2.63	b	17.50	b

FAUR	GP		GI		TGS	
	Mean	LSD test	Mean	LSD test	Mean	LSD test
C	98.75	a	2.96	a	19.75	a
D1	92.50	abc	2.78	abc	18.50	abc
D2	95.00	abc	2.85	abc	19.00	abc
D3	97.50	ab	2.93	ab	19.50	ab
D4	87.50	cd	2.63	cd	17.50	cd
D5	93.75	abc	2.81	abc	18.75	abc
D6	88.75	bcd	2.66	bcd	17.75	bcd
D7	81.25	d	2.44	d	16.25	d

MIRANDA	GP		GI		TGS	
	Mean	LSD test	Mean	LSD test	Mean	LSD test
C	97.50	a	2.93	a	19.50	a
D1	97.50	a	2.93	a	19.50	a
D2	95.00	a	2.85	a	19.00	a
D3	93.75	ab	2.81	ab	18.75	ab
D4	93.75	ab	2.81	ab	18.75	ab
D5	97.50	a	2.93	a	19.50	a
D6	96.25	a	2.89	a	19.25	a
D7	85.00	b	2.55	b	17.00	b

TRANSILVANIA	GP		GI		TGS	
	Mean	LSD test	Mean	LSD test	Mean	LSD test
C	90.00	ab	2.70	ab	18.00	ab
D1	90.00	ab	2.70	ab	18.00	ab
D2	95.00	ab	2.85	ab	19.00	ab
D3	96.25	a	2.89	a	19.25	a
D4	87.50	abc	2.63	abc	17.50	abc
D5	85.00	bc	2.55	bc	17.00	bc
D6	86.25	abc	2.59	abc	17.25	abc
D7	78.75	c	2.36	c	15.75	c

Figure 1. Mean values and the least significant difference for germination percentage (GP), germination index (GI), and total germinated seeds (TGS) assessed in the fourth day of the germination of all the sixteen wheat varieties subjected to control (C), D1-100 mM NaCl, D2-125 mM NaCl, D3-150 mM NaCl, D4-175 mM NaCl, D5-200 Mm NaCl, D6-225 mM NaCl and D7- 250 mM NaCl. ANOVA: Alex (F=14.06; p<0.001), Andrada (F=15.57; p<0.001); Bezostaia (F=15.99; p<0.001); Boema (F=6.42; p<0.05); Arieșan (F=10.85; p<0.005); Ciprian (F=24.92; p<0.001); Esențial (F=14.82; p<0.001); Faur (F=13.05; p=0.001); Glosa (F=12.94; p=0.001); Granny (F=5.54; p<0.05); Fundulea (F=30.39; p<0.001); Miranda (F=4.46; p<0.05); Otilia (F=0.52; p<0.5); Pădureni (F=17.89; p<0.001); Taisa (F=1.67; p<0.5); Transilvania (F=7.29; p<0.05)

Conducting the experiment up to the seventh day did not lead to major differences in the values distribution of the three parameters, but about 56% of the varieties did not record significant decreases in their values with increasing salt doses (Figure 2). This may indicate a tendency for Alex, Andrada, Glosa, Bezostaia, Granny, Fundulea, Otilia, Taisa and Transilvania to adapt to the treatments applied. Some of the varieties respectively Alex, Boema, Esențial, Faur, Pădureni and Transilvania had a more successful germination process compared with the control, in some of the variants, with a maximum recorded in the 100-200 mM NaCl concentration range. The general trend was to record the minimum at the most concentrated saline solution, D7 (250 mM NaCl), an aspect characteristic to about 69% of the varieties. Two of them, respectively Glosa and Fundulea performed minimally both below the highest dose and below D3 while Taisa performed weekly below D5.

The variability outlined by the recorded results suggests that the germination process progression, associated with germination percentage (GP), fluctuates in response to both saline dose and wheat variety (Pastuszak et al., 2022). Certain studies indicate that germination can be initiated even at 300 mM, which accounts for the trends observed in several varieties. However, subsequent development on the BBCH scale would no longer be feasible due to the adverse effects on cell division (Munns and James, 2003).

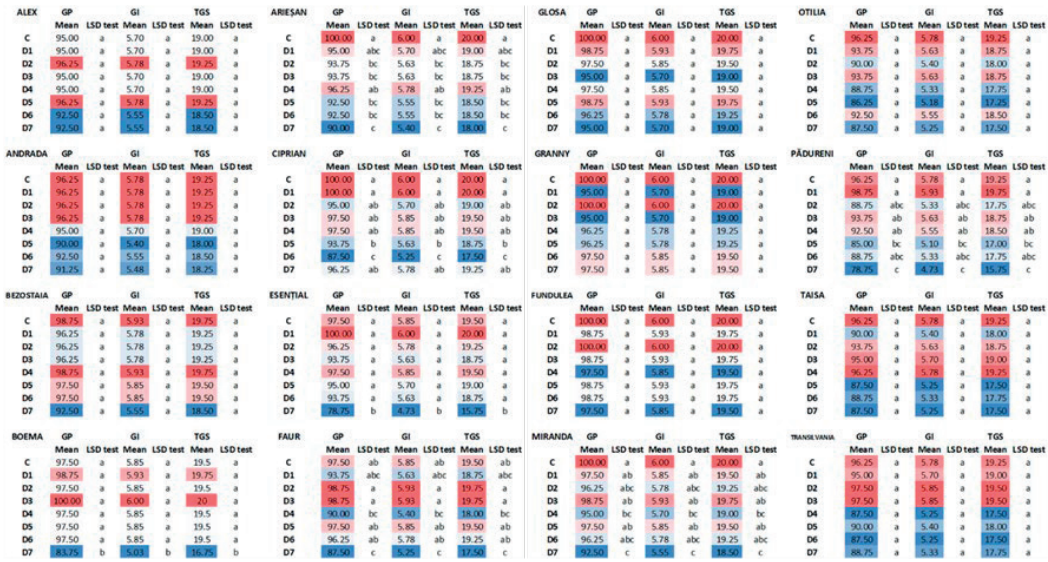


Figure 2. Mean values and the least significant difference for germination percentage (GP), germination index (GI), and total germinated seeds (TGS) assessed in the seventh day of the germination of all the sixteen wheat varieties subjected to control (C), D1-100 mM NaCl, D2-125 mM NaCl, D3-150 mM NaCl, D4-175 mM NaCl, D5-200 Mm NaCl, D6-225 mM NaCl and D7- 250 mM NaCl. ANOVA: Alex (F=0.78; p=0.385), Andrada (F=2.82; p=0.103); Bezostaia (F=0.86; p=0.360); Boema (F=6.63; p<0.05); Arieșan (F=11.43; p<0.005); Ciprian (F=13.05; p=0.001); Esențial (F=12.22; p=0.001); Faur (F=3.75; p<0.1); Glosa (F=2.95; p<0.1); Granny (F=0.33; p=0.572); Fundulea (F=2.62; p=0.116); Miranda (F=9.93; p<0.005); Otilia (F=2.36; p=0.135); Pădureni (F=15.45; p<0.001); Taisa (F=3.10; p<0.1); Transilvania (F=7.18; p<0.05)

The Relative Salt Injury Rate assessment

The parameter performance RSIR varies with the wheat variety, but also with the applied saline dose. Plants salt injury mechanism is complex and influences plant growth and development throughout their lifespan by disrupting various processes starting with germination inhibition (Hao et al., 2021) (Figure 3). The determined value of the parameter also varies with the time exposure to salt stress and the degree of seedling development at the time of measurement. After analysing the data, the impact of salt stress is stronger on the fourth day and tends to decrease with the time and the development of the seedlings. The Andrada variety shows the most interesting aspect, i.e. the plants seem to have an equal or lower RSIR compared to the control up to and including D4, on day 4. On contrast, longer exposure (day 7) to stress decreases RSIR under all saline doses. This may be associated with an adaptation of the plants to the tested doses. At the same time, another interesting aspect was outlined in the varieties Ariesan, Boema and Faur, as well as in the wheat variety Otilia, where D1 had a positive effect, reducing the RSIR both on the fourth day and especially on the seventh day. The outlined aspects highlight a temporal adaptation of certain wheat varieties to certain saline doses. This is due to the wheat plants adaptability through various mechanisms that operate throughout the entire plant organism, starting at cellular level (El Sabagh et al., 2021). A totally opposite aspect was observed at Alex, Bezostaia, Ciprian, Esential and Fundulea, where under D1, the three days' transition increased the RSIR. This shows that, although initially the application of the dose may be stimulating, the effect is diminished to approximately equal or lower performances compared with the control. The RSIR is also obviously stronger in Glosa, Granny and Taisa varieties. RSIR evolution indicates Andrada as the most tolerant wheat in the tested germplasm collection.

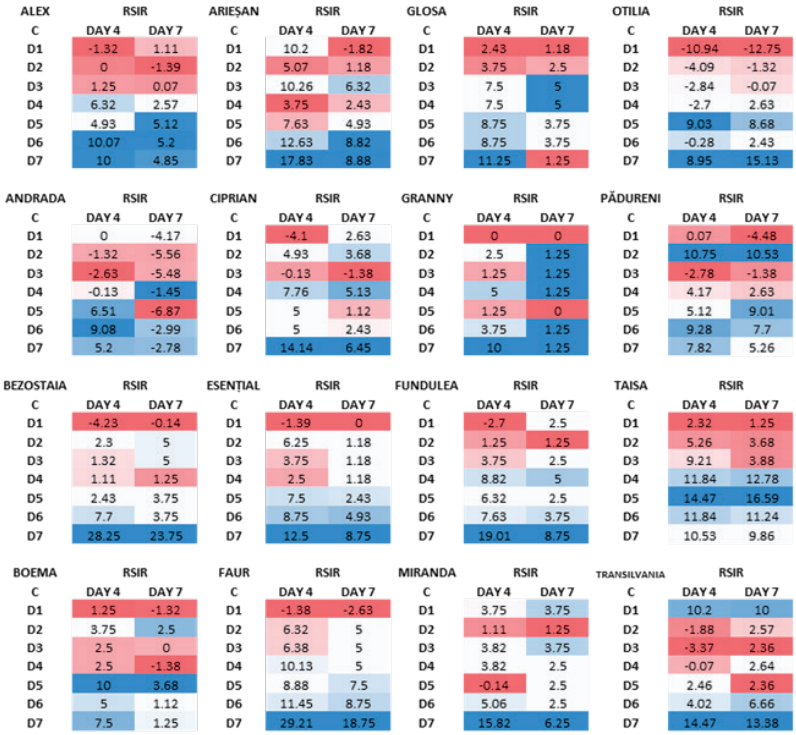


Figure 3. Mean values of relative salt injury rates (RSIR) assessed in the fourth and the seventh day of the germination of all the sixteen wheat varieties subjected to control (C), D1-100 mM NaCl, D2-125 mM NaCl, D3-150 mM NaCl, D4-175 mM NaCl, D5-200 Mm NaCl, D6-225 mM NaCl and D7-250 mM NaCl

The impact of salinity on BBCH development

The plant development on the BBCH scale was influenced by the saline doses. The trend outlined on the fourth day reveals that increasing salt treatment concentrations delayed the seedling growth process. More than half of the varieties reached the highest level of BBCH development, respectively BBCH 10 (Figure 4). Lower rates were observed for the varieties Alex, Andrada, Bezostaia, Boema, Esențial, Faur and Miranda. The development up to BBCH 09 under the influence of D4 dose reflects an increased tolerance of Taisa and Pădureni varieties compared to the variants at all the other doses. Transilvania, Otilia, Fundulea, Granny, Ciprian and Boema were ranked with lower tolerance than the two varieties. An interesting aspect is outlined in Bezostaia, where doses more concentrated than D3 gradually decrease the growth in the fourth day assessment. Most seedlings of this variety range between BBCH 05 and BBCH 07. Another interesting aspect was evidenced in Alex, where a few seedlings were still in BBCH 03 on day 4, at the doses D4, D6 and D7. This indicates a sensitivity of the germination process to high saline doses for this variety. These aspects highlighted the sensitivity of early growth and developmental stages, as defined by the BBCH scale, to salt stress. This sensitivity arises from the osmotic stress and toxicity induced by saline concentrations (El Sabagh et al., 2021), which adversely affect cell division and elongation processes (Terletskaya et al., 2020), particularly during the early growth phases (Baranova and Gulevich, 2021).

The saline doses effect on seedling growth and development is also evident on the seventh day of germination (Figure 5).

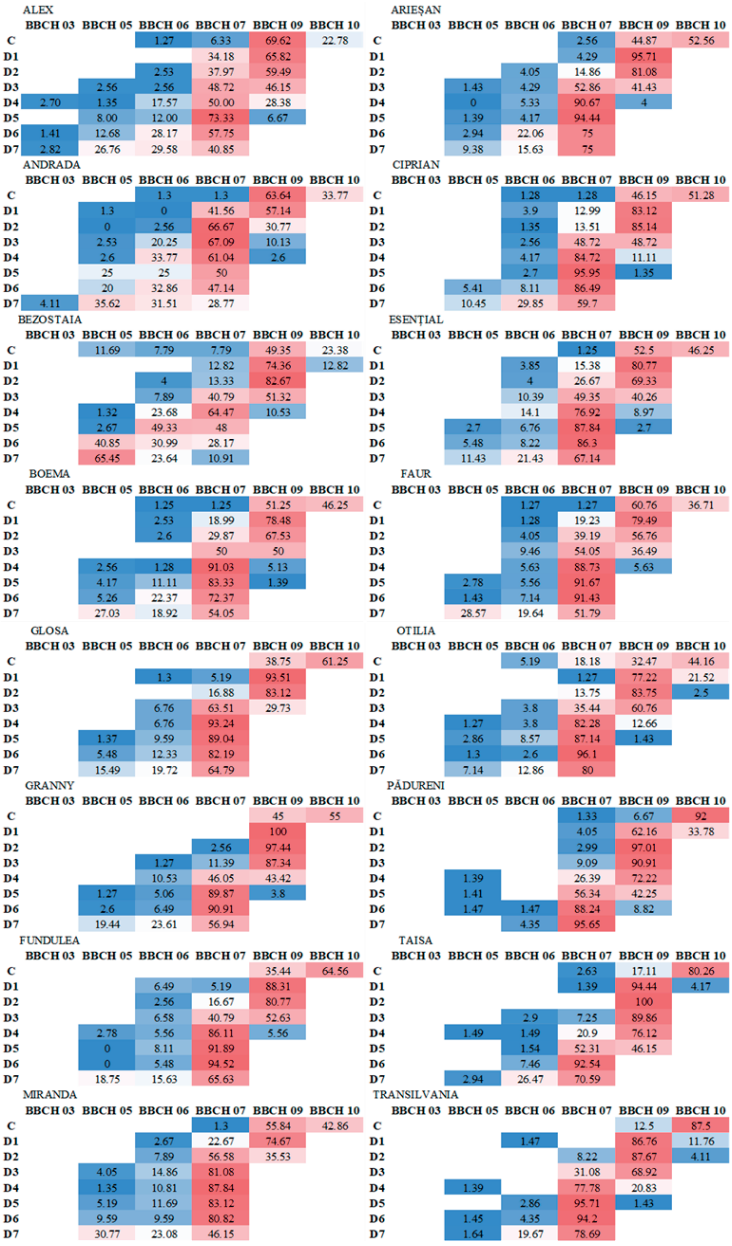


Figure 4. Percentage distribution of BBCH growth stages in the fourth day of germination for all the sixteen wheat varieties subjected to control (C), D1-100 mM NaCl, D2-125 mM NaCl, D3-150 mM NaCl, D4-175 mM NaCl, D5-200 Mm NaCl, D6-225 mM NaCl and D7-250 mM NaC

The most developed growth was seen in the control plants of the Glosa, Granny, Fundulea, Otilia, Pădureni, Taisa and Transilvania varieties, which already had an unfolded leaf. The tolerance of Taisa wheat to salinity was highlighted, at doses from D1 to D4, showed the same degree of development, with seedlings in BBCH 10. Transilvania, Pădureni and Fundulea, varieties whose seedlings, although performing better than other varieties, grew up to BBCH 10 when tested at D3 and lower doses, were significantly less tolerant than Taisa.

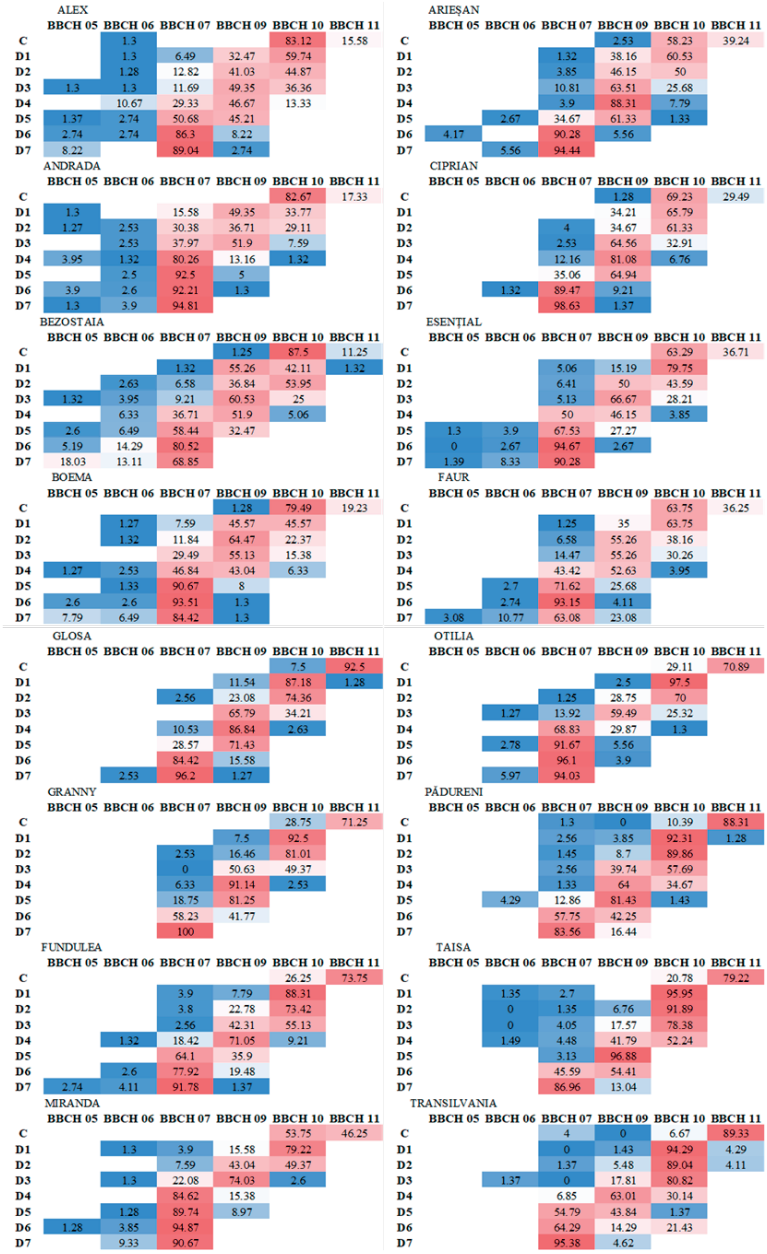


Figure 5. Percentage distribution of BBCH growth stages in the seventh day of germination for all the sixteen wheat varieties subjected to control (C), D1-100 mM NaCl, D2-125 mM NaCl, D3-150 mM NaCl, D4-175 mM NaCl, D5-200 Mm NaCl, D6-225 mM NaCl and D7-250 mM NaC

Among the growth delayed seedlings were those of the Alex and Bezostaia varieties, which under higher concentrations of D3 reached maximum BBCH 07. With even lower performance than these, Andrada and Boema reached maximum BBCH 07 at higher concentrations than D4. The high dose of salt stress affects cell division and growth, which is why germination is delayed or even stopped (El Sabagh et al., 2021).

CONCLUSIONS

By monitoring and evaluating germination through the analyzed parameters, it was observed that the earlier stage of germination was more sensitive to salt stress. Additionally, the temporal evolution of the parameters is associated with a tendency for seedlings to adapt to lower saline concentrations in most of the tested varieties.

The RSIR evaluation at two distinct stages of germination revealed an adaptive response over time to saline stress, reflected in the reduced impact. Furthermore, the Andrada wheat variety performed well under saline conditions, while Taisa, Transilvania, and Pădureni experienced negative effects in the presence of saline stress, marked by an increase in RSIR.

The progression along the BBCH scale was significantly influenced by the saline stress application, particularly at higher concentrations. Prolonged exposure to the highest saline concentration until the seventh day of germination indicated a trend of reduced tolerance across all varieties, with most achieving a maximum development stage of only BBCH07.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

DIVA-GIS APPROACH TO DIVERSITY ANALYSIS OF *Aegilops cylindrica* GERMPLASM

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Abstract

The object of the study was 152 specimens of Aegilops cylindrica Host. collected in 2024 from 17 districts in Bulgaria. DIVA-GIS approach was used to analyze the diversity. The descriptive analysis of the studied quantitative characteristics showed a wide range of variability among accessions. The Shannon diversity index exhibited a high level of diversity, with a maximum range of 1.109-2.0, 1.109-2.0, and 0.879-2.0, respectively, for the traits of plant height, spike length, and number of spikelets per spike. These findings indicate a notable responsiveness of these traits to the ecosystem. The grid maps generated for the diversity analysis of these characters indicated the occurrence of diverse accessions for plant height and for spike length from the municipality of Elin Pelin (district Sofia), and for number of spikelets per spike from the Kyustendil (district Kyustendil), Belitca and Bansko (district Blagoevgrad), Sevlievo (district Gabrovo), Karlovo (district Plovdiv), Mineralni bani (district Haskovo) and Elin Pelin (district Sofia) municipalities. The study would facilitate more effective management and utilization of Aegilops cilindrica Host. in the country.

Key words*: Aegilops cylindrica Host., diversity, expedition, Diva-Gis.*

INTRODUCTION

The generation, analysis and use of data on PGR is critical to their effective and rational management and can significantly increase the value of genetic resources. Many of these data are geo-referenced and can be analyzed using Geographical Information Systems (GIS) - and links can be made between them and other geo-referenced data from sources external to the plant genetic resources process (Garcia et al., 2017; Bagarinao, 2022).

The accurate determination and collection of plant material constitutes a pivotal step in the efficient conservation and utilization of available germplasm. DIVA-GIS is a methodology designed to support plant genetic resources and biodiversity communities in mapping the geographical distribution of species of interest (Hijmans et al., 2002). This analysis is imperative for identifying potential areas of biodiversity and collection gaps, which must be taken into consideration during the design of subsequent collection missions (Mujaju & Fatih, 2011; Suma et al., 2019; Reshmi et al., 2022). Projected distribution maps for each taxon, based on climatic data, can identify gaps in *ex situ* conservation, both in regions where the species' seeds have not been collected, or where the species has not been sufficiently studied (Shehadeh et al., 2013; Garcia et al., 2017).

DIVA-GIS has been demonstrated to offer an efficacious means of evaluating diversity loss, thereby facilitating the formulation of effective strategies for the conservation and utilization of genetic resources in a perpetually evolving global context. GIS mapping has been successfully used over the years for assessing biodiversity and in identifying areas of high diversity (Chand et al., 2018)

The analysis of ecogeographic data has been demonstrated to be a valuable tool in the study of the habitat requirements of specific groups of plant species. Furthermore, this analysis has been shown to facilitate a more comprehensive understanding of the sympatric distributions of taxa within a given gene pool (Maxted et al., 2011, 2015).

The presence of genetic diversity within maintained or collected materials is of paramount importance to any crop

improvement program. A comprehensive understanding of the extent of genetic variability is essential for the judicious utilization of germplasm (Suma et al., 2019). Identification of potential donor(s) for yield, combined with desired quantitative and qualitative traits, is a prerequisite for the success of breeding programs. In order to ensure the preservation of allelic and genotypic variability within a species, it is imperative to collect a representative sample of genetically diverse populations. These populations should be studied and, when feasible, retained for the purpose of breeding improvement (Raina et al., 2013).

The aim of the study was to analyze the diversity of the collected jointed goatgrass germplasm in terms of some quantitative traits using DIVA-GIS approach.

MATERIALS AND METHODS

In the period June - August 2024, expeditions were carried out to identify the natural localities of the wild relative of wheat - *Aegilops cylindrica* Host. in 17 districts of the territory of the Republic of Bulgaria, as well as to collect seed material for storage in the National Genebank. A route method was used to conduct the expeditions, and seed material was collected from established and marked locations at the stage of full plant maturity using the method described by Porceddu and Damania (1994).

Bioversity Multi-Crop passport descriptors (Alercia et al., 2012) including: institute code; temporary registration number for registration in the PHYTO 2000 database; international registration number in the EURISCO electronic database; taxonomic affiliation - genus, species; biological status; geographical coordinates - location (nearest locality), altitude, latitude, longitude were used to generate passport data of marked and collected material. A handheld geographical positioning system (GPS-Garmin 12) was used to record geographical coordinates. Additionally *in situ* assessment was carried out by analyzing the following quantitative traits viz. plant height, spike length and number of spikelets per spike, and qualitative traits viz. color of the spike and the awns, hairness of the spike and the awns.

DIVA-GIS version 7.5.0 software elaborated by Hijmans et al. (2005) was utilised for the purpose of determining the spatial distribution. This was achieved by converting point data into a grid analysis using simple circular neighbourhood methods. The diversity analysis for quantitative traits viz. plant height, spike length and number of spikelets per spike was then calculated using the Shannon–Weaver diversity index (Hijmans et al., 2000; 2012; Shankar et al., 2023). For the analysis of country-level data, a grid of 90 × 90 km cells was utilised for the allocation of points to grid cells, with the objective of mapping the diversity of germplasm for the studied traits (Hijmans et al., 2000).

RESULTS AND DISCUSSIONS

A total of 152 accessions were collected from 136 localities, covering 17 districts of Bulgaria (Table 1, Figure 1). The recorded geographical coordinates of the marked sites vary widely, with latitude and longitude ranging from 41°23'-43°20'N and 22°32'-28°03', respectively. The altitudes at which the specimens were found ranged from 2 to 1185 metres above sea level (Table 1). The map for the distribution of accessions according to altitudinal range showed that most accessions had been collected at 0 to 554 m, following at 554 to 1110 m above sea level (Figure 2). Zaharieva et al. (2004) notes that *Aegilops cylindrica* Host. is found in nearly all ecological zones in Bulgaria, but is more common in the northern part of the country. In this study, the largest number of specimens was recorded in the Veliko Tarnovo district located in northern Bulgaria. A considerable number of accessions were also marked and collected in seven provinces (between 11 and 16 number of accessions). Expeditions have also confirmed that the species is most often to be found in uncultivated and highly disturbed areas, e.g. wastelands, roadsides, dry, sandy, grassy slopes, grasslands (Valchinova et al., 2019; Sanchez, 2022). The collection sites mapped are provided in Figure 1.

Table 1. *Aegilops cylindrica* Host. collected during several germplasm expeditions in 2024 on the territory of Bulgaria

District	No. of municipality	No. of habitats	No. of collected accessions	Geographical coordinates (range)		
				Latitude (N)	Longitude (E)	Altitude
Pazardzhik	3	12	15	42°07'-42°30'	24° 07'-24°56'	227-570
Plovdiv	6	13	16	41°51'-42°48'	24°37'-25°54'	189-1185
Lovech	1	5	6	43°04'-43°08'	24 °44'-24°53'	199-488
Gabrovo	2	4	5	42°46'-42°59'	25°5'-25 °18'	207-950
Stara Zagora	6	16	16	42°24'-42°42'	24 °46'-25°48'	237-552
Kardzhali	1	3	3	41°43'-41°47'	25°14'-25°21'	402-563
Blagoevgrad	6	12	16	42°02'-41°29'	23°29'-23°44'	244-306
Burgas	6	12	16	42°25'-42°62'	27°6'-27°15'	2-344
Smolyan	2	3	3	41°23'-42°30'	24°48' -25°05'	444-702
Haskovo	2	5	5	41°49'-42°00'	25°19'-25°23'	221-388
Yambol	1	1	1	42°32'	26°29'	146
Sofia	5	16	16	42°04'-42°43'	23°35'-24°01'	494-811
Veliko Tarnovo	4	19	19	42°40'-43°08'	25°18'-25°45'	50-326
Varna	2	2	2	42°58'-43°08'	27°18'-27°26'	103-111
Dobrich	1	1	1	43°20'	28°03'	5
Pleven	1	1	1	42°08'	24°15'	186
Kyustendil	3	11	11	42°13'-42°18'	22°32'-25°02'	454-938
17	**52**	**136**	**152**	41°23'-43°20'	22°32'-28°03'	2-1185

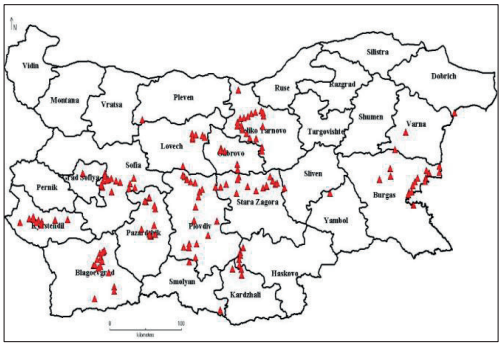


Figure 1. DIVA-GIS distribution mapping of collection sites of *Aegilops cylindrica* Host. in Bulgaria during 2024

Diva -GIS has been successfully applied to identify specific characteristics of samples collected from diversity-rich areas, thereby increasing the the likelihood of identifying ideal genotypes and saving the resources and time needed to evaluate large numbers of accessions. This facilitates faster use of germplasm in breeding programmes (Dar et al., 2016; Suma et al., 2019; Reshmi, 2022).

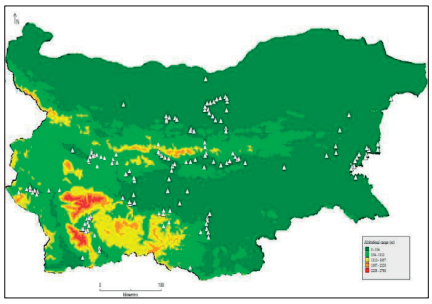


Figure 2. Distribution of collected accessions of *Aegilops cylindrica* Host. in Bulgaria according to altitude using DIVA-GIS

A wide range of variability was evident for the quantitative traits among the 152 accessions, as indicated by the wide range of Shannon diversity index for plant height (0.000-0.277 to 1.109-2.000), spike length (0.000-0.277 to 1.109-2.000) and spikelets per spike (0.000-0.220 to 0.879-2.000) (Figures 3-5).

The grid map generated for the diversity analysis of the plant height showed that one grid cell (shown with red color) with the highest Shannon diversity index between 1.109 and 2.000 was located in the municipality of Elin Pelin (district Sofia), while 9 grid cells (orange color in the map) with high Shannon index between 0.832 and 1.109 were located in different provinces. Two grid cells were identified in Plovdiv (Sopot and Karlovo municipalities) and in Blagoevgrad districts (Belitza and Bansko municipalities), one in Kyustendil (Kyustendil municipality), Sofia (Elin Pelin municipality), Lovech (Lovech municipality), Gabrovo (Sevlievo municipality), Stara Zagora (Maglizh municipality) (Figure 3).

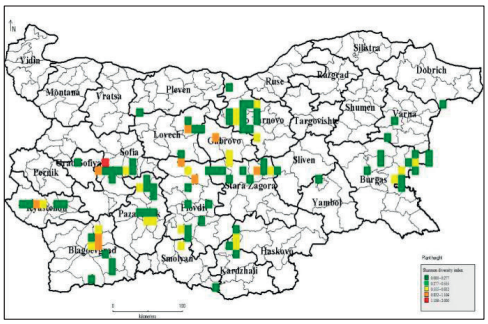


Figure 3. Diversity map of collected jointed goatgrass germplasm for the plant height in 90 × 90 km grid cells using the Shannon Diversity Index (H)

The diversity map for spike length revealed that the region with the highest diversity (H=1.109-2.000), was also in the municipality of Elin Pelin (district Sofia). Generated grid cells with high Shannon index (0.832-1.09) were in nine municipalities, respectively in 7 districts of the country - Kyustendil municipality (Kyustendil district), Bansko municipality (Blagoevgrad district), Panagyuriste municipality (Pazardzhik district), Karlovo, Sopot and Rodopi municipalities (Plovdiv district), Sevlievo municipality (Gabrovo district), Maglizh municipality (Stara Zagora district) and Mineralni bani municipality (Haskovo district) (Figure 4).

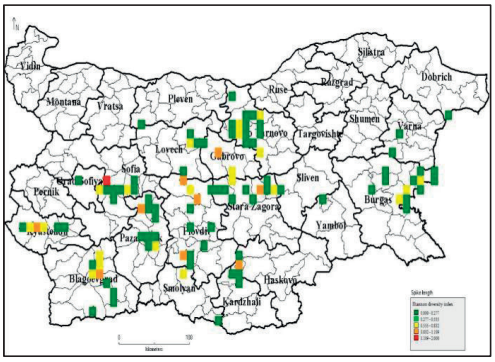


Figure 4. Diversity map of collected jointed goatgrass germplasm for the characteristic spike length in 90 × 90 km grid cells using the Shannon Diversity Index (H)

The diversity grid map generated for the number of spikelets per spike showed that nine grid cells with the highest Shannon diversity index between 0.897 and 2.000 were located in the following districts - Kyustendil (one cell in Kyustendil municipality), Sofia (two cells in Elin Pelin municipality), Blagoevgrad districts (one cell in Belitza and one cell in Bansko municipalities), Gabrovo (one cell in Sevlievo municipality), Plovdiv (one cell in Sopot and one cell in Karlovo municipality) and Haskovo (one cell in Mineralni bani municipality). Fifteen grid cells with Shannon index between 0.659 and 897 were deployed in nine districts, four cells in Veliko Tarnovo, two cells in each of the following provinces - Blagoevgrad and Plovdiv. The districts in which one orange cell was generated in Sofia, Pazardzhik, Plovdiv, Smolyan, in the border of Haskovo and Kardzhali, Stara Zagora and Burgas (Figure 5).

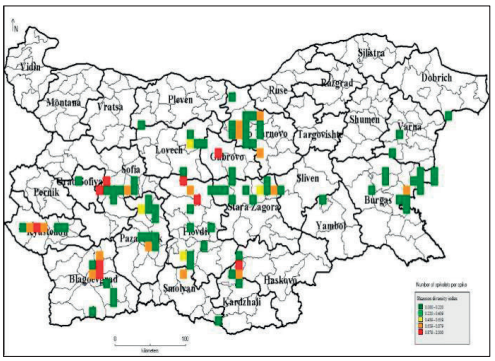


Figure 5. Diversity map of collected jointed goatgrass germplasm for the number of spikelets per spike in 90 × 90 km grid cells using the Shannon Diversity Index (H)

Awareness of the variation in morphology, its nature and extent, is vital to the choice of the appropriate genotypes for successful use in breeding programmes. The valuable resource of germplasm is dependent not only on the quantity of specimens, but also on the genetic variability among them (Kumar et al., 2013). The descriptive analysis of the studied quantitative characteristics showed a wide range of variability among the 152 accessions (Table 2). Plant height fluctuated between 16 and 80 cm. Spike length varied between 3.5 and 18 cm, while the number of spikelets per spike ranged between 4 and 10. The highest variability was found for plant height, where the calculated coefficient of variation (CV, %) was 30.336%.

Table 2. Descriptive statistical analysis of quantity characteristics for 152 accessions of *Aegilops cylindrica* Host

Traits	Min.	Max.	Mean	Standard deviation	Vari Ance	Coefici ent of variation %
Plant height, cm	16	80	43.467	13.186	173. 872	30.336
Spike length, cm	3.5	18	11.117	2.903	8.427	26.114
Number of spikelets per spike	4	10	6.563	1.268	1.608	19.32

The highest CV between 42% and 53% was recorded for accessions from Belica and Bansko municipality (Blagoevgrad province), Sevlievo municipality (Gabrovo province) and Polski Trambesh (Veliko Tarnovo province). The high CV of 31-42% was found in six municipalities filling in five districts (Figure 6).

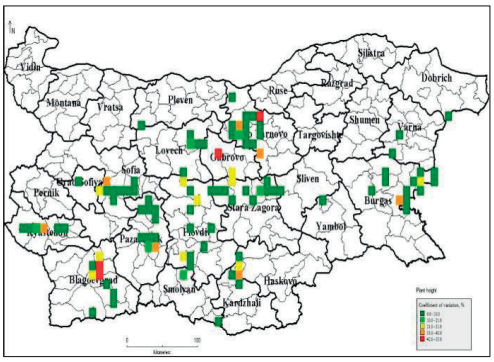


Figure 6. Grid map of coefficient of variation for plant height generated with DIVA-GIS

Germplasm accessions augmented from Sevlievo municipality (Gabrovo province) recorded the highest CV (54-69%) for spike length, from Chepelare municipality (Smolyan) and Karlovo municipality (Plovdiv) high CV (41-54%), while medium CV (27-41%) from one municipality in Blagoevgrad district (Figure 7).

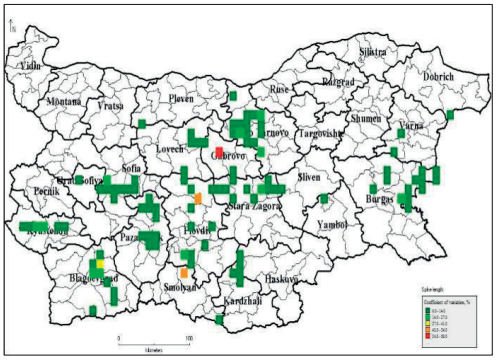


Figure 7. Grid map of coefficient of variation for spike length generated with DIVA-GIS

The accessions with the maximum CV (27-34%) recorded for the number of spikelets per spike originated from six municipalities, falling in five districts. The generated grid map identifies 76 grin cells with low CV (0-7%), indicating less variability for the accessions collected from these localities (Figure 8).

The present study revealed that diverse germplasm accessions of *Aegilops cylindrica* Host were dispersed all over the 17 districts, but regions where the diverse genotypes most likely to found were Sofia, Kyustendil, Blagoevgrad, Gabrovo, Plovdiv, Stara Zagora, where high diversity indices and CV were recorded for the studied traits. These areas should be considered for future exploration programs to capture maximum diversity for these characters.

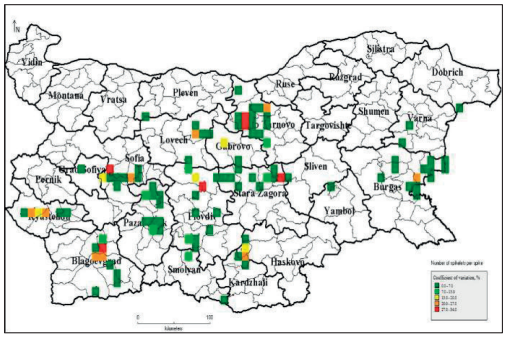


Figure 8. Grid map of coefficient of variation for number of spikelets per spike generated with DIVA-GIS

CONCLUSIONS

In this study, the diversity of jointed goatgrass germplasm collected in 2024 for three quantitative traits - plant height, spike length and number of spikelets per spike - was investigated using the DIVA-GIS tool, which helped to identify the regions with the greatest diversity for the traits studied. Future collection missions should focus on the following regions - Sofia, Plovdiv, Blagoevgrad, Kyustendil, Gabrovo, Stara Zagora - to capture maximum diversity for these and other traits. The study using DIVA-GIS should continue with the analysis of collected and preserved specimens of *Aegilops* in the National Collection to identify conservation gaps, prioritise areas for the establishment of genetic reserves. Further research is needed to predict distribution in other parts of the country and to assess loss of diversity as part of a future conservation strategy and sustainable use.

ACKNOWLEDGEMENTS

This work was financially supported by the Bulgarian National Science Fund, Ministry of Education and Science under the project КП-06-ПН76/3 “Study of the genetic diversity of *Aegilops* species in the flora of Bulgaria”.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

GENETIC CONTROL AND COMBINING ABILITY IN LINE BY TESTER CROSSES OF COTTON

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Abstract

The aim of this study was to evaluate the nature of gene action, combinnig ability of parents and F_1 hybrids in line × tester cotton crosses. An approach was applied to identify the parents having as high as possible GCA for all studied traits and to select the most reliable F_1 crosses by their ranking based on the total effects using the squared Euclidean distance. Crosses with relatively short Euclidean distances to the "ideal" population in which desirable segregats are expected to occur were identified. The crosses 572 × Chirpan-539, Melani × Chirpan-539 and 641 × Chirpan-539 had the highest positive GCA for the three studed traits and appeared to be the most valuable for breeding programs. Heterosis manifestations were found for the three traits in individual croses, the heterobeltiosis for productivity per plant ranged from 103.7% to 141.9%, for lint percentage was from 101.3% to 107.2% and for fiber length - from 102.2% to 106.8%. Good general combiners were identified and high GCA of some was mainly due to additive genes, making them very suitable for the synthetic selection.

Key words*: cotton, G. hirsutum, genetic variance, heterosis, Euclidean distance.*

INTRODUCTION

In our country, in cotton breeding, through intra- and interspecific hybridization and experimental mutagenesis, a number of new varieties and selection lines have been created. Their usage as starting material in breeding programs is of great importance for increasing the genetic potential for yield and fiber quality of new cotton varieties.

The main components of genetic variance - additive and non-additive, genetic parameters and indicators such as degree of dominance and type of inheritance, number of effective factors, heritability in broad and narrow sense, heterosis, etc., and the combining ability of parents for hybridization are of importance for clarifying the selection strategy.

Based on the estimates of genetic variance additive or non-additive recommendations are made for effective selection in the earlier or later hybrid generations. In the inheritance of traits, both additive and non-additive variance may be relevant. In the presence of additivity the prediction for selection in the early segregating generations can be good.

Many authors have reported that additive and non-additive gene effects are important for inheritance of traits in cotton. Some researchers have pointed greater importance of additive (Ekinci & Basbag, 2018; Vasconcelos et al., 2018; Carvalho et al., 2018; Bourgou et al, 2023), others of non-additive gene effects (Ali et al., 2018; Munir et al., 2018; Ullah et al., 2022).

According to Sivia et al. (2017), Nimbal et al. (2019) analysis of combining ability plays an important role in the selection of parents to produce more desirable segregants. General combining ability (GCA) is due to additive gene action, which is very important for faster selection, while specific combining ability (SCA) is caused by non-additive gene action - dominance and epistasis.

Barut et al. (2000) used an approach to select parents having general combining ability as high as possible for all traits. The total GCA effects of parents, for each trait and for each cross combination, were pooled and ranked, and the highest value for each trait was taken as "ideal". The "ideal" values for each trait were considered to characterize the "ideal" population and the Euclidean distances between the GCA effects of any two parents and the "ideal" population values for each F_1 cross were calculated. Crosses with the shortest

Euclidean distance, i.e. closest to the "ideal" population, were considered to be most valuable.

The aim of this study was: i) to determine the type of gene action controlling the investigated traits in line × tester cotton crosses in relation the choice of selection strategy and work with the hybrid generations; ii) to evaluate the general and specific combining abilities of parents and by applying the approach mentioned above to identify the parents having general combining ability as high as possible for all studied traits and to select the most reliable F_1 crosses.

MATERIALS AND METHODS

Fifty six cotton hybrid populations derived from two line × tester crosses design mating systems by applying the experimental method I (without reciprocals) of Savchenko (1984) were the objective of this study. In the first crossing scheme (7 × 3) four advanced lines 449, 489, 572, 578 and three varieties Anabel, Tiara and Selena (Bulgarian selection) used as females were crossed with Sirius variety and the cultivars Chirpan-539 (Bulgarian) and FR-H-1002 (Spanish) used as males to produced 21 hybrid combinations. In the second crossing scheme (7 × 5) other five promising lines 639, 641, 643, 681, 682 and Aida and Melani varieties (Bulagrian) used as females were crossed with Sirius variety and the cultivars Chirpan-539, Helius (Bulgarian), FR-H-1002 (Spanish) and Nazili 954 (Turkish) used as males to produced other 35 hybrid combinations.

The trials were carried out in 2020 at the Field Crops Institute in Chirpan, in randomized complete block design with three replications. Each plot with parents and hybrids consisted of two rows 2.4 m long with a distance of 0.60 m between rows and 0.20 m within row. Traditional practices for cotton growing in our country were applied during the growing season. The traits under study were productivity per plant, lint percentage and mean fiber length. For each genotype ten plants from each replication were observed.

General combining ability (GCA) and specific combining ability (SCA) were evaluated by applying the methodology of Savchenko (1984). The main effects of females and males are equivalent to GCA, and the female × male interaction represents SCA (Hallauer & Miranda, 1981). To rank the parents on GCA for the three studied traits total effects of females and males ($f_i + m_j$) were calculated for each trait. The highest ($f_i + m_j$) values were taken as "ideals" (Barut et al., 2000) and the Squared Euclidean Distances (Manly, 1995) were calculated between those "ideals" and total effects of any two parents for each F_1 hybrid combination. The shorter the Euclidean Distances to the "ideal" population, the more desirable the crosses are. Euclidean distances were determined by the formula:

$$\text{Squared Euclidean Distance} = \sum(x_i - x_j)^2$$

where: x_i - "ideal" value;

x_j - total GCA effects of any two parents.

The studied traits were measured in different units and data were pre-standardized.

RESULTS AND DISCUSSIONS

Analysis of combining ability variance (Table 1) reveals that effects of crosses, GCA effects of females and males, and SCA effects of crosses are significant for all three traits in both crossing schemes, which means that females and males differed in general and specific combining ability. Only GCA effects of females for fiber length in the first crossing scheme (Trial I) were insignificant.

The participation of σ^2_{GCA} and σ^2_{SCA} in the genetic variance of productivity per plant reveals that non-additive gene effects were essential for the inheritance of this trait in both crossing schemes. The results obtained are consistent with those reported by Çoban & Ünay (2017), Munir et al. (2018), Roy et al. (2018), Ali et al. (2018), Ullah et al. (2022), Vadodariya et al. (2022) that non-additive gene action predominated in inheritance of productivity per plant and seed cotton yield in intra- and interspecific (*G. hirsutum* × *G. barbadense*) crosses. Regarding inheritance of lint percentage additive gene effects predominated $\sigma^2_{GCA} > \sigma^2_{SCA}$ in the first group of crosses, while non-additive gene effects have had predominance $\sigma^2_{GCA} < \sigma^2_{SCA}$ in the second

group of crosses. Preponderance of additive gene action in inheritance of this trait was reported by Vasconcelos et al. (2018), Vadodariya (2022) in *G. hirsutum* diallel and *G. hirsutum* × *G. barbadense* cotton crosses. In contrast, other researchers Ali et al. (2018) and Munir et al. (2018), Ullah et al. (2022) in intra- and interspecific cotton crosses reported non-additive gene action in the inheritance of lint percentage. As for fiber length, in the first group of crosses, the values of variance components σ^2_{GCA} and σ^2_{SCA} are close and reveal that additive and non additive gene effects were of importance in inheritance of this trait. In the second group of crosses, non-additive gene effects prevailed over additive gene effects $\sigma^2_{GCA} < \sigma^2_{SCA}$, which is consistent with that found by Khan et al. (2017) (in *G. hirsutum* crosses), Munir et al. (2018) (in intra- and interspecific crosses), Ali et al. (2018) (in line × tester crosses), Roy et al. (2018), Vadodariya et al. (2022) (in *G. hirsutum* × *G. barbadense* crosses), that non-additive gene effects were more important in fiber length inheritance. Superiority of additive gene effects for fiber length was reported by Ekinci & Basbag (2018) in interspecific *G. hirsutum* × *G. barbadense* crosses, Carvalho et al. (2018), Bourgou et al. (2023) in *G. hirsutum* diallel crosses. Crosses from the first group demonstrated more additive gene action for lint percentage and fiber length, which from a selection point of view is important for rapid improvement of this trait. These results confirm that the choice of parents can increase additivity. In the second group of crosses the studed traits were influenced by non-additive gene action and quick selection regarding these traits cannot be expected. The three traits were strongly influenced by year conditions. Vozhehova et al. (2022) found that year conditions affected the adaptive capacity of cotton samples. Muhova & Stefanova-Dobreva (2022) studed the effect of mineral fertilization as well as the influence of year conditions on the seed cotton yield.

Table 1. Analysis of combining ability variance for productivity per plant, lint percentage and fiber length in 7 × 3 (Trial I) and 7 × 5 (Trial II) F_1 line × tester crosses

Sources on variation	Degrees of freedom	Mean squares		
		Productivity per plant	Lint percentage	Fiber length
Trial I				
Crosses	20	120.303 $F_{exp.}$ 18.143***	3.450 $F_{exp.}$ 24.126***	1.258 $F_{exp.}$ 4.642***
Errors	60	6.631	0.143	0.271
GCA-females	6	33.544 $F_{exp.}$ 15.178**	0.395 $F_{exp.}$ 8.229**	0.163 $F_{exp.}$ 1.811 ns
GCA-males	2	93.424 $F_{exp.}$ 42.273**	0.226 $F_{exp.}$ 4.708*	1.410 $F_{exp.}$ 15.667**
SCA-females × males	12	34.489 $F_{exp.}$ 15.606**	1.681 $F_{exp.}$ 35.021**	0.382 $F_{exp.}$ 4.244**
Errors	60	2.210	0.048	0.090
Components of variance		σ^2_{GCA}=0.146; σ^2_{SCA}=10.760	σ^2_{GCA}=1.328; σ^2_{SCA}=0.544;	σ^2_{GCA}=0.093; σ^2_{SCA}=0.097;
Trial II				
Crosses	34	165.446 $F_{exp.}$ 72.279***	19.048 $F_{exp.}$ 84.283***	3.197 $F_{exp.}$ 24.977***
Errors	92	2.289	0.226	0.128
GCA-females	6	124.620 $F_{exp.}$ 163.329**	28.514 $F_{exp.}$ 380.187**	2.315 $F_{exp.}$ 53.837**
GCA-males	4	72.012 $F_{exp.}$ 94.380**	5.016 $F_{exp.}$ 66.880**	0.593 $F_{exp.}$ 13.791**
SCA-females × males	24	34.970 $F_{exp.}$ 45.832**	1.030 $F_{exp.}$ 13.733**	0.832 $F_{exp.}$ 19.349**
Errors	92	0.763	0.075	0.043
Components of variance		σ^2_{GCA}=0.394; σ^2_{SCA}=23.750	σ^2_{GCA}=0.109; σ^2_{SCA}=0.318;	σ^2_{GCA}=0.005; σ^2_{SCA}=0.263;

*, ** and *** at P 0.5%, 0.1% and 0.01%

Evaluations of GCA effects are presented in Table 2. Of the females included in the first crossing scheme (Trial I), Tiara variety having the lowest productivity per plant showed the highest GCA followed by Anabel variety and line 449, the last one having the highest productivity per plant. Among the males, the cultivar Chirpan-539 showed the highest productivity per plant had the highest GCA. This cultivar and line 449 could be used as good general combiners in breeding programs to enhence productivity of new cotton varieties. Of the females included in the second crossing scheme (Trial II) high GCA was found for Melani variety and lines 639 and 641 having low to medium high productivity per plant. Lines having high productivity per plant exhibited low GCA. Of the males, the Turkish cultivar Nazili 954 had the highest GCA. The cultivar Helius and Sirius variety having high productivity per plant had lower GCA. Sirius variety having the highest productivity among the males in the first group of crosses showed negative GCA. The Spanish cultivar FR-H-1002 also had inconsistent GCA. It is necessary the GCA of both varieties to be evaluated in different crosses and in different environments. The cultivars Nazili 954 and Helius could be used as good general combiners for productivity in breeding programs with cotton.
Of the females in the Trial I, line 572 having the lowest lint percentage had the highest GCA for this trait. Selena variety also having low lint percentage had negative but insignificant GCA. Other lines having the same lint percentage had different combining abilities. Of the males, the cultivar Chirpan-539 showed the highest lint percentage had negative but insignificant GCA, the Spanish cultivar FR-H-1002 had positive GCA but lower lint percentage.

Table 2. Estimates of general combining ability (GCA) effects of parents for productivity per plant, lint percentage and fiber length

Parents		Characters					
		Productivity per plant		Lint percentage		Fiber length	
		g	GCA	%	GCA	mm	GCA
Trial I							
Females	449	59.4	1.616	36.9	-0.368	26.5	-0.060
	489	54.8	-5.017	36.5	0.365	25.2	0.162
	Anabel	49.5	1.794	36.6	0.054	25.2	0.040
	Tiara	39.8	5.149	36.5	-0.079	25.9	-0.249
	572	40.9	-0.784	35.5	0.554	25.4	0.095
	578	42.3	-2.984	36.2	-0.446	25.5	0.340
	Selena	41.7	0.226	35.9	-0.079	25.5	-0.327
	Standard error		1.487		0.218		0.301
Males	Chirpan-539	44.9	3.221	38.1	-0.194	25.6	0.508
	Sirius	43.1	-3.970	35.5	0.030	25.0	-0.344
	FR-H-1002	39.9	0.749	36.2	0.163	26.0	-0.163
	Standard error		0.973		0.143		0.197
	Trial II						
Females	Aida	50.9	-1.025	37.3	0.973	26.3	-0.584
	Melani	37.9	4.652	37.1	0.849	26.7	-0.150
	639	43.0	6.16	40.5	2.663	25.4	-0.610
	641	34.0	4.388	35.2	1.535	26.1	-0.210
	643	37.4	-5.825	35.1	0.702	28.5	-0.337
	681	56.3	-4.832	33.6	-3.447	28.7	1.163
	682	56.6	-3.518	32.2	-3.275	26.2	0.729
	Standard error		0.675		0.213		0.159
Males	Chirpan-539	46.3	-3.085	38.0	0.879	28.1	0.434
	Helius	49.3	0.865	37.6	-0.560	25.2	-0.318
	Sirius	54.3	0.961	36.8	-0.086	25.6	-0.156
	Nazili 954	46.6	4.448	36.4	-1.051	27.2	0.129
	FR-H-1002	46.1	-3.190	36.9	0.818	28.1	-0.089
	Standard error		0.573		0.180		0.135

Of the females in the Trial II, line 639 had the highest lint percentage and the highest GCA. This line could be used in breeding programs for improving of this trait. Four other lines exibited positive GCA, but their lint percentage was very low. Among the males, the cultivar Chirpan-539 had the highest lint percentage and the highest GCA.

Regarding fiber length, in the first group of crosses, line 578 from the females and the cultivar Chirpan-539 from the males showed the best general combining abilities. Insignificant positive GCA was obsereved for Anabel variety and lines 489 and 572. In the second group of crosses, these were line 681 from the females and the cultivar Chirpan-539 from the males exhibited the highest general combining abilities and the longest fibers. Among the males, the cultivars Chirpan-539 and FR-H-1002 having equal and the longest fibers demonstrated positive and negative GCAs, respectively. The cultivar Chirpan-539 showed the highest positive and significant GCA was followed by the Turkish cultivar Nazili 954, while the Spanish cultivar FR-H-1002 had insignificant negative GCA. The cultivar Helius and Sirius variety formed the shortest fibers and had negative GCAs.

Analysis of results reveals that the parents having the same mean values for the studied traits exhibited different GCAs. Genotypes with high mean values did not always develop high GCA. According to Batool et al. (2010), Makhdoom et al. (2010) many commercial cultivars regardless of how they perform economically, better or worse, when combined in crosses can perform differently better or worse.

In Trial I nine crosses demonstrated positive and significant SCA effects for productivity per plant, highest in Anabel × FR-H-1002, Tiara × Chirpan-539 and 572 × Chirpan-539 (Table 3). Of these ones, eight crosses showed heterosis relative to average of the two parents (mid parent heterosis) from 106.8% to 150.4%, seven crosses relative to the better parent (heterobeltiosis) from 105.8% to 141.9%. The crosses Anabel × FR-H-1002 and Tiara × Chirpan-539 had the highest productivity per plant and the highest mid parent heterosis of 143.2% and 150.4%, and heterobeltiosis of 129.3% and 141.9%. Tiara variety and line 449

Table 3. Mean values, effects of specific combining ability (SCA) (below line) and variances (σ^2S_i; σ^2S_j) for the first group of crosses included in the Trial I

Females	Productivity per plant, g				Lint percentage, %				Fiber length, mm			
	Males											
	Chirpan-539	Sirius	FR-H-1002	σ^2S_j	Chirpan-539	Sirius	FR-H-1002	σ^2S_j	Chirpan-539	Sirius	FR-H-1002	σ^2S_j
449	57.5	47.0	50.6		37.1	36.0	38.0		26.5	24.4	24.3	
	2.579	-0.730	-1.849	5.092	0.249	-1.075	0.825	0.944	0.936	-0.311	-0.625	0.674
489	46.4	44.2	44.6		36.3	38.2	38.7		26.0	24.5	25.3	
	-1.89	3.103	-1.216	7.124	-1.284	0.459	0.825	1.266	0.181	-0.340	0.219	0.112
Anabel	53.2	38.2	64.1		37.2	36.9	38.2		24.7	25.4	25.4	
	-1.79	-9.708	11.51	114.7	-0.04	-0.563	0.603	0.340	-0.963	0.555	0.408	0.693
Tiara	63.7	49.8	52.2		38.8	37.4	35.7		25.5	24.5	24.6	
	5.246	-1.463	-3.783	21.78	1.694	0.037	-1.73	2.927	0.159	-0.055	-0.103	0.011
572	54.3	50.4	43.2		36.8	38.2	38.8		25.4	25.2	25.0	
	1.779	5.070	-6.849	37.68	-0.973	0.237	0.736	0.768	-0.286	0.367	-0.081	0.103
578	45.4	45.6	50.3		35.6	37.5	37.7		26.5	25.0	24.8	
	-4.92	2.470	2.451	17.95	-1.140	0.560	0.560	0.970	0.570	-0.111	-0.459	0.265
Selena	52.5	47.6	50.8		38.6	37.7	35.6		24.7	24.4	25.3	
	-0.99	1.259	-0.260	1.114	1.494	0.337	-1.83	2.842	-0.570	-0.044	0.641	0.376
σ^2S_j	11.35	23.11	33.89		1.505	0.358	1.485		0.429	0.111	0.199	
SE (SCA)	1.863				0.273				0.375			

from the females and the cultivar Chirpan-539 from the males having high GCA effects had low variances of SCA, which means that their high GCA was mainly due to additive gene action and they are very suitable for hybridization and selection programs. Anabel variety (as female component) having high GCA has manifested high variances of SCA, which means that its high GCA was due to additive and non-additive gene actions.

Ten crosses exhibited positive SCA effects for lint percentage, the highest in Tiara × Chirpan-539 and Selena × Chirpan-539. All crosses having positive SCA effects manifested mid parent heterosis from 104.0% to 108.1% and heterobeltiosis from 101.3% to 107.2%. The crosses Tiara × Chirpan-539, 489 × FR-H-1002 and 572 × FR-H-1002 had the highest lint percentage of 38.7-38.8% and evaluated heterobeltiosis was 101.8%, 106.0% and 107.2%, respectively. Lines 572 of females, had low variance of SCA and is very suitable for the pedigree selection, while line 489 of females and the cultivar FR-H-1002 of males showed high GCA had high SCA variances and are more suitable for the heterosis selection.

Regarding fiber length, only four crosses showed positive and significant SCA effects. Of these ones, only two crosses manifested very weak mid parent heterosis of 101.2-101.5%. Line 578 of the females and the cultivar Chirpan-539 of the males showed high GCAs for this trait exhibited low and high variances of SCA, respectively. The cross 449 × Chirpan-539 having the longest fiber manifested very low mid parent heterosis of 101.5%.

In Trial II nine crosses showed positive SCA effects for productivity per plant (Table 4). Heterosis manifestations were registered in six crosses. The values of mid parent heterosis ranged from 107.0% to 144.4%, of heterobeltiosis - from 103.7% to 148.5%. The cross 641 × Nazili 954 showed the best productivity performance, followed by the cross 641 × Sirius both exhibited heterobeltiosis of 148.5% and 117.3%, respectively. Melani variety and line 639 from the females and the cultivar Helius from the males having high GCA had low SCA variances. Line 641 from the females possessed high GCA and high SCA variance. The cultivar Nazili 954 and Sirius variety from the females having high GCA had high and medium-high SCA variances, respectively. At low SCA variances, high GCA is due to additive gene action, while at high and medium-high SCA variances, high GCA is due to additive and non-additive gene action, which should be considered in recommendations for selection of productivity.

Heterosis results for productivity per plant in this study are comparable to those reported by other authors. Positive mid parent heterosis for this trait in the hybridization of the species *G. hirsutum* × *G. barbadense* was reported by Adsare et al. (2017), Rajeev et al. (2018). Tian et al. (2019) concluded that overdominance is the genetic basis of lint yield heterosis in intra- and interspecific hybrids. Borzan & Güvercin (2021) noted that F_1 hybrids can be more superior from mean of parents (mid parent heterosis) and superior parent (heterobeltiosis) because of the influence of dominant genes. Tian et al. (2019), Li et al. (2022) noted that heterosis is related to overdominance.

Positive and significant SCA effects for lint percentage were recorded in 9 crosses. Mid parent heterosis from 3.5% to 4.4% was observed in three crosses, heterobeltiosis from 3.0% to 6.0% was registered in only two crosses, which is consistent with the study of Karademir & Gençer (2010) who reported heterosis level of 5.52% in only one cross among studied diallel crosses. The crosses 639 × Chirpan-539 and 639 × FR-H-1002 had the highest lint percentage of 40.0%, respectively with significant and insignificant positive, but not very high SCA effects. In these two crosses, the female component also exhibited high lint percentage of 40.5%. Line 639 from the females having high GCA showed low SCA variance, the cultivar Chirpan-539 from the males, with high GCA had medium high SCA variance. This cultivar is suitable for both pedigree selection and heterosis selection.

As for fiber length, positive SCA effects were found in 9 crosses. Heterosis relative to the mean of both parents from 101.5% to 109.5% was registrated in all nine crosses, while heterobeltiosis from 102.2% to 106.8% was reported in only 4 crosses.

Table 4. Mean values, effects of specific combining ability (SCA) (below line) and variances (σ^2S_i; σ^2S_j) for the second group of crosses included in the Trial II

Females	Males					
	Chirpan-539	Helius	Sirius	Nazili 954	FR-H-1002	σ^2S_i
Productivity per plant, g						
Aida	35.6	52.8	56.3	59.9	43.8	
	-11.008	2.242	5.678	5.792	-2.703	49.740
Melani	52.6	58.8	56.4	61.3	47.6	
	0.315	2.715	0.068	1.482	-4.580	7.575
639	53.3	59.2	56.6	58.9	56.4	
	-0.493	1.456	-1.248	-2.427	2.711	4.204
641	52.0	48.4	63.7	69.2	42.2	
	-0.022	-7.572	7.665	9.645	-9.717	75.795
643	43.5	46.6	39.8	45.7	48.9	
	1.692	0.842	-6.055	-3.675	7.196	26.294
681	48.7	47.6	46.2	42.1	44.8	
	5.932	0.848	-0.648	-8.235	2.103	27.053
682	47.7	47.5	42.7	49.1	49.0	
	3.585	-0.532	-5.461	-2.582	4.990	18.544
σ^2S_j	28.650	12.173	26.491	37.019	35.107	
SE(SCA)	2.654					
Lint percentage, %						
Aida	38.2	35.2	37.5	36.0	37.9	
	0.368	-1.202	0.628	0.135	0.071	0.491
Melani	36.0	37.3	38.2	35.7	37.1	
	-1.687	1.005	1.432	0.013	-0.763	1.614
639	40.0	36.7	38.5	37.8	40.0	
	0.649	-1.295	0.014	0.250	0.381	0.568
641	40.1	37.3	35.8	36.5	37.7	
	1.673	0.428	-1.546	0.057	-0.612	1.428
643	36.8	37.9	35.9	35.2	37.5	
	-0.694	1.811	-0.670	-0.476	0.059	1.112
681	33.4	32.6	32.5	31.1	33.2	
	-0.003	0.589	-0.005	-0.494	-0.088	0.141
682	33.3	30.8	32.7	32.2	34.4	
	-0.305	-1.337	0.177	0.513	0.952	0.732
σ^2S_j	1.121	1.613	0.884	0.128	0.329	
SE(SCA)	0.385					
Fiber length, mm						
Aida	27.6	27.1	26.4	26.2	26.0	
	0.545	0.764	-0.097	-0.616	-0.597	0.402
Melani	30.0	25.3	26.2	27.2	26.7	
	2.479	-1.435	-0.730	0.017	-0.330	2.207
639	25.5	26.4	27.3	26.5	27.4	
	-1.560	0.091	0.863	-0.256	0.863	0.995
641	26.7	26.5	26.8	27.5	27.7	
	-0.794	-0.175	-0.104	0.310	0.763	0.333
643	26.3	27.5	27.3	27.5	25.9	
	-1.068	0.918	0.556	0.470	-0.877	0.816
681	28.5	28.1	28.1	28.6	28.6	
	-0.301	0.051	-0.110	0.103	0.256	0.041
682	29.1	27.4	27.4	28.1	27.8	
	0.699	-0.215	-0.377	-0.029	-0.077	0.166
σ^2S_j	1.867	0.591	0.289	0.124	0.434	
SE(SCA)	0.290					

The cross Melani × Chirpan-539 exhibited the longest fiber of 30.0 mm and the highest heterobeltiosis, followed by the cross 682 × Chirpan-539 having fiber length of 29.1 mm and manifested heterobeltiosis of 103.5%. In the other crosses exhibited positive SCA effects

fiber length varied from 27.1 mm to 27.7 mm. had low SCA variances, while the cultivar Chirpan-539 from the males showed high GCA had high variance of SCA.

Çoban et al. (2015), Çoban & Ünay (2017) in *G. hirsutum* × *G. barbadense* crosses reported mid parent heterosis for fiber length in all cross combinations. Heterosis values were positive and ranged between 0.34% to 14.33% and 2.05% to 16.99% (Çoban et al., 2015). Bölek et al. (2014), Roy et al. (2018), Ünay et al. (2018) also reported positive heterosis for fiber length in this hybridization. According to Ekinci & Basbag (2018) among the studied *G. hirsutum* × *G. barbadense* crosses the determined heterosis (Ht) values for fiber length varied from -1.77 to 3.81%, the estimated heterobeltiosis (Hb) values for this trait varied from -7.87% to -0.03%. Chapara & Madugula (2021) reported heterosis for Upper Half Mean Length (UHML) and mean length in intraspecific line × tester crosses and the highest values were 21.34% and 25.37%, heterobeltiosis for UHML ranged between 7.53 to 20.73% which was higer than achieved in this study.

Line 681 from the females having high GCA Total GCA effects (f_i+m_j) of the parents for each trait and the ranked crosses based on the Euclidean distances to the "ideal" population are presented in Tables 5 and 6. According to the calculated Euclidean distances, in Trial I the cross 572 × Chirpan-539 was the closest to the "ideal" population and most valuable for selection programs. The parental components line 572 and the cultivar Chirpan – 539 had positive total (f_i+m_j) GCA effects for the three traits. The crosses Tiara × Chirpan-539, Tiara × FR-H-1002, Anabel × Chirpan-539 and 449 × Chirpan-539 had high total GCA effects for productivity per plant, but showed negative effects for lint percentage or fiber length (at the second cross). In these crosses, with increasing productivity decrease in lint percentage and fiber length could be expected. Crosses 572 × FR-H-1002 and 572 × Sirius having high GCAs for lint percentage showed negative ones for productivity per plant and fiber length. The crosses 578 × Chirpan-539 and 489 × Chirpan-539 having the highest positive effects for fiber length showed negative effects for lint percentage and productivity per plant.

Table 5. Total general combining ability (GCA) effects ($f_i + m_j$) of the parents for each trait and Squared Euclidean Distances to the "ideal" population in the first group of crosses - Trial I

Parents	Total GCA (general combining effects) of the parents ($f_i + m_j$)			Squared Euclidean Distance
	Productivity per plant, g	Lint percentage, %	Fiber length, mm	
572 × Chirpan-539	2.437	0.360	0.602	3.033
Anabel × Chirpan-539	5.015	-0.140	0.547	6.254
489 × Chirpan-539	-1.796	0.171	0.668	7.605
572 × FR-H-1002	-0.035	0.717	-0.068	8.061
Anabel × FR-H-1002	2.543	0.217	-0.123	8.487
Tiara × Chirpan-539	8.370	-0.273	0.258	8.77
Selena × Chirpan-539	3.447	-0.273	0.180	10.536
Tiara × FR-H-1002	5.898	0.084	-0.412	11.521
489 × FR-H-1002	-4.268	0.528	-0.002	12.289
449 × Chirpan-539	4.837	-0.562	0.447	13.068
449 × FR-H-1002	2.365	-0.205	-0.223	13.921
Selena × FR-H-1002	0.975	0.084	-0.49	15.093
578 × FR-H-1002	-2.235	-0.283	0.177	15.244
572 × Sirius	-4.754	0.584	-0.249	15.343
Anabel × Sirius	-2.176	0.084	-0.304	15.563
578 × Chirpan-539	0.237	-0.64	0.847	16.444
Tiara × Sirius	1.179	-0.049	-0.593	17.767
489 × Sirius	-8.987	0.395	-0.183	21.860
449 × Sirius	-2.354	-0.338	-0.404	22.069
Selena × Sirius	-3.744	-0.049	-0.671	23.883
578 × Sirius	-6.954	-0.416	-0.004	25.011

In the Trial II, the crosses Melani × Chirpan-539 and 641 × Chirpan-539 were found to be most reliable, with the shortest Euclidean distances and positive total GCA effects of parents for the three traits. Crosses 639 × Nazili 954 and 641 × Nazili 954 had the highest total GCA effects for productivity per plant, crosses 639× Chirpan-539 and 639 × FR-H-1002 for lint percentage, but all showed negative GCA effects for fiber length. In the first two crosses with increasing productivity and in the second two with increasing lint percentage shortening of fiber length could be expected. The crosses 681 × Nazili 954 and 682 × Chirpan-539 exhibited the highest total GCA effects for fiber length and negative effects for productivity per plant and lint percentage. According to Naoumkina et al. (2019) inproving fiber lenth without reduction yield is one of major goals of cotton breeding. In cotton, there are strong negative dependencies between many of the traits (Gospodinova et al., 2020).

Table 6. Totals general combining ability (GCA) effects ($f_i + m_j$) of the parents for each trait and Squared Euclidean Distances to the "ideal" population in the second group of crosses - Trial II

Parents	Productivity per plant, g	Lint percentage, %	Fiber length, mm	Squared Euclidean Distance
Melani × Chirpan-539	1.568	1.728	0.284	6.871
641 × Chirpan-539	1.304	2.414	0.224	7.008
641 × Nazili 954	8.836	0.484	-0.081	7.653
Melani × Nazili 954	9.1	-0.202	-0.021	8.041
639 × Chirpan-539	3.076	3.542	-0.176	8.435
639 × Nazili 954	10.608	1.612	-0.481	9.692
641 × Sirius	5.349	1.449	-0.366	9.744
Melani × Sirius	5.613	0.763	-0.306	9.765
Melani × FR-H-1002	1.462	1.667	-0.239	10.419
641 × FR-H-1002	1.198	2.353	-0.299	10.675
641 × Helius	5.253	0.975	-0.528	11.556
Melani × Helius	5.517	0.289	-0.468	11.65
639 × Sirius	7.121	2.577	-0.766	12.242
Aida × Nazili 954	3.423	-0.087	-0.455	12.842
639 × FR-H-1002	2.97	3.481	-0.699	12.940
Aida × Chirpan-539	-4.109	1.852	-0.15	13.999
639 × Helius	7.025	2.103	-0.928	14.124
643 × Nazili 954	-1.377	-0.349	-0.208	14.22
682 × Nazili 954	0.93	-4.326	0.858	15.226
681 × Nazili 954	-0.384	-4.498	1.291	15.659
682 × Sirius	-2.557	-3.361	0.573	16.356
682 × Chirpan-539	-6.602	-2.396	1.163	16.387
Aida × Sirius	-0.064	0.887	-0.74	16.414
681 × Sirius	-3.871	-3.533	1.006	16.514
643 × Chirpan-539	-8.909	1.581	0.097	17.891
681 × Chirpan-539	-7.916	-2.568	1.596	17.904
682 × FR-H-1002	-6.708	-2.457	0.64	18.157
681 × Helius	-3.967	-4.007	0.844	18.293
682 × Helius	-2.653	-3.835	0.411	18.392
643 × Sirius	-4.864	0.616	-0.493	18.515
Aida × FR-H-1002	-4.215	1.791	-0.673	18.534
Aida × Helius	-0.16	0.413	-0.902	18.609
681 × FR-H-1002	-8.022	-2.629	1.073	18.739
643 × Helius	-4.96	0.142	-0.655	20.618
643 × FR-H-1002	-9.015	1.52	-0.426	21.925

CONCLUSIONS

Crosses with relatively short Euclidean distances to the "ideal" population in which desirable segregants are expected to occur were identified.

The crosses 572 × Chirpan-539, Melani × Chirpan-539 and 641 × Chirpan-539 had the highest positive GCA for the three studed traits and appeared to be the most valuable for breeding programs.

The approach of identifying parents with the highest possible GCA for all studed traits and ranking the parental means using the squared Euclidean distance emerged as an effective method for selecting the most reliable F_1 crosses.

Some crosses showed high positive SCA effects and heterosis. Heterosis manifestations were better expressed in the second group of crosses where heterobeltiosis for productivity per plant ranged from 103.7% to 148.5%, for lint percentage it was from 103.0% to 106.0% and for fiber length - from 102.2 % to 106.8%.

Line 449 of the females from the first group of crosses was identified as good general combiner for productivity, lines 639 and 681 of the females from the the second croup of crosses emerged as good general combiners for lint percentage and fiber length, respectively. These parents have distinguished with high means for the respective traits, high GCAs and low SCA variances, which make them very valuable for hybridization and breeding programs.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

RESPONSE OF MAIZE BREEDING TO CLIMATE CHANGE

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Abstract

Maize is the species with highest world production and the most widely planted agricultural crop. Breeders and the agricultural community around the world will have to expand the capacity of current breeding programs to increase yield potentials in the conditions of climate changes. In the paper the effect of key factors such warmer temperatures, changes in rainfall and higher concentrations of CO2 on the productivity of maize were reviewed based on the literature. The most sensitive stages to drought were shown. Breeding tools to mitigate unfavorable effect of climate changes on grain yield of maize were reviewed. Breeding program achievements of the Maize Research Institute Knezha, Bulgaria was shown. New hybrids Kneja 461 and Kneja 561 due to their drought tolerance were found as more productive comparing to the standards Kneja 435 and Kneja 509 in new conditions. Future prospects of the breeding of maize were shown as development of maize varieties resistant to high temperatures; selection and improvement of maize varieties capable of maintaining growth and productivity under changed environment, ect.

Key words*: climate change, drought, maize.*

INTRODUCTION

Maize is the species with highest world production and as the most widely planted agricultural crop plays an irreplaceable role in ensuring food security (Ranum et al., 2014). Given the rapidly expanding global population, addressing the food security needs and the rising demand for food and also feed has become increasingly crucial (Ifeanyi, 2024) Climate change (in particular increased temperatures and drought) is acutely related to food security of humans, which strongly depends on the growth of crops as maize, wheat, and rice.

When considering the net impacts of climate on food production, we need to consider three key factors: warmer temperatures, changes in rainfall (which can cause too much, or not enough, water) and higher concentrations of CO_2. Meteorological factors during the growing season of maize are of big importance for the yield. In recent decades, noticeable climate changes have been observed, expressed in an increase in the active temperature sum and uneven precipitation (Huang et al., 2015; Qiao et al., 2025).

Grain maize is projected to be the most affected crop by climate change in Europe. According to Tesfaye et al. (2018) under fully irrigated conditions, substantial yield reductions are estimated for most producing countries, being more severe in Southern Europe in all scenarios. Maize loses around 15% of its global yield due to drought stress (Tesfaye et al., 2018). Therefore, to prevent these yield losses and to maintain maize yields in dry regions, the drought tolerance of maize must be improved. An important aspect related to weather conditions is the crop yield. Yield can vary from year-to-year, as a result of ever changing natural conditions, especially the weather. Identifying high-yielding and sustainable genotypes in diverse environments has been a constant challenge for plant breeders worldwide.

Create new varieties with increased resistance to drought and heat stress with high yield potential and stable productivity over the years, a result of mitigating the negative impact of adverse weather conditions. Considering all of the above our work aimed at to assess the response of maize hybrids to meteorological conditions vis. temperature and rainfall in a given location.

MATERIALS AND METHODS

The experiment was carried out at the experimental field of the Maize Research Institute - Knezha, Bulgaria under non-irrigated

conditions during four consequently years (2020- 2023). Four hybrids from two maturity groups were tested as follows: mid-early hybrids (FAO 400-500) Kneja 435 and Kneja 461, and mid-late hybrids (FAO 500-599) Kneja 509 and Kneja 561. A block method was used, 4 replications, the size of the experimental plot was 10 m2 and the density of 5300 plants/da. The soil type was typical chernozem. The standard technology of growing of maize was applied. Meteorological conditions (rainfall and daily average temperature of growing seasons were recorded.

In addition, the meteorological conditions for long term period (1991-2020) was shown. Hydrothermal Coefficient (HTC) was calculated for the reproductive stage (sum of precipitation and accumulated sum of daily temperature from last decade of June and all days of July) for each year of study. To calculate the Hydrothermal coefficient the next formula as suggested Selyaninov was applied: HTC= Σ x / Σ t x 10; where Σ x and Σ t – accordingly sum of precipitations and temperatures in the period. Interpretation of the hydrothermal coefficient according to Selyaninov was based on the following: HTC from 1.0 till 2.0 – humidity is sufficient; HTC > 2.0 – immoderately humid; HTC < 1.0 – insufficient humidity; HTC from 1.0 till 0.7 – dry; HTC from 0.7 till 0.4 – very dry. The grain yield was calculated in kg/da and it was reported at moisture content of 14%. Factorial regression was performed to obtain a biological explanation of the hybrid, environment, and hybrid - environment interaction for the yield.

Statistical analysis of the experimental data using Anova without and with replication processing was performed using the SPSS 20.0 software product for Windows.

RESULTS AND DISCUSSIONS

The meteorological conditions comprising the growing season of maize over a long-term period (30 years) are presented in Figure 1. From the data, it can be seen that the mean daily temperature increases gradually from April to July and August. The most significant temperature difference was found between April and May (4.1°C), while between May, June and July it was about 3°C. The highest monthly mean daily temperatures were recorded in the typically summer months of July and August, with values very similar.

Monthly rainfall totals increase from the beginning of the growing season in April to July, again with the most significant difference between April and May (about 14 mm), while between May, June and July (65, 67 and 70 mm), the difference ranged from 2 to 5 mm. A significant decrease in the rainfall total for this long-term average period was recorded in August (37 mm), with a total for the growing season of 289.0 mm.

For the 2020-2023 study period, meteorological conditions during the maize growing season by year are presented in Figure 2. A gradual increase in mean daily temperature occurs from April to July and August. In May and June the temperature was above 16°C and above 20°C respectively, with higher values of 17.4°C and 21.6°C only in 2022. Comparing the differences of the monthly mean daily temperatures of April and May over the long-term period with those of the study years, the differences were found to increase from 4.1°C to 5.0°C, 6.0°C, 6.2°C and 5.0°C for 2020, 2021, 2022 and 2023, respectively. This accelerated warming at the beginning of the growing season in four consecutive years is indicative of a change in the environmental conditions during the vegetative stage of crop development. Temperatures were found highest in July, with only 2020 recording a higher value for August (23.5°C). The high temperatures in July coincide with the reproductive stage of maize development, which can have an extremely negative effect on the yield as suggested by Shiduo et al. (2024) also.

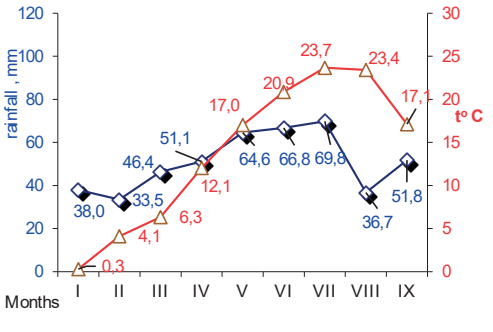


Figure 1. Meteorological conditions including the growing seasons of maize for the long term 30 years period 1991-2020

The precipitation was very irregularly distributed by months. The highest quantities average for the period were registered in May and June and the lowest in July (Table 1).

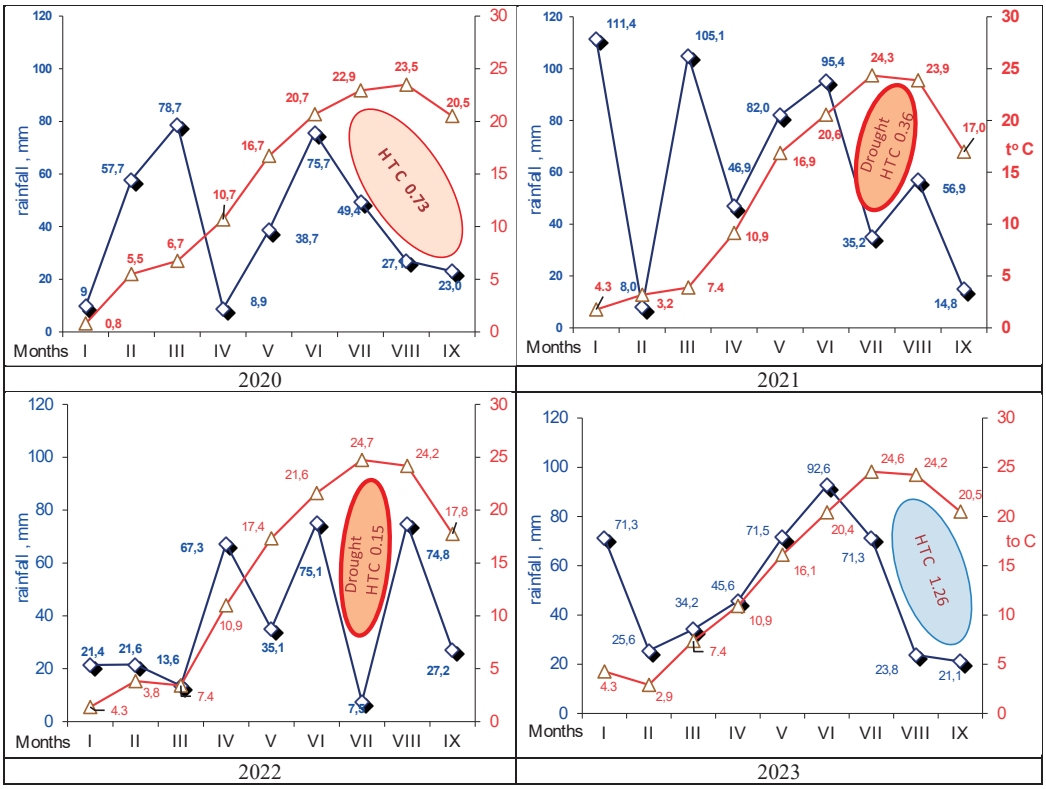


Figure 2. Meteorological conditions during the growing seasons of maize for the period 2020-2023

The total precipitation amounts for the growing season fluctuated by year. The smallest amount was recorded in 2020 being 26% less than the average for the period. The highest precipitations as a sum were recorded during years 2021 and 2023, exceeding the average for the period by 17 and 13%, respectively.

Table 1. Precipitation for the growing season by years, mm

Year/months	2020	2021	2022	2023	Average
IV	8.9	46.9	67.3	45.6	42.2
V	38.7	82.0	35.1	71.5	56.8
VI	75.7	95.4	75.1	92.6	84.7
VII	49.4	35.2	7.5	71.3	40.9
VIII	27.1	56.9	74.8	23.8	45.7
∑precipitation	199.8	316.4	259.8	304.8	270.2
HTC	0.73	0.36	0.15	1.26	

Undoubtedly, precipitation is the most important factor influencing on the maize grain yield (Huang et al., 2015). How this precipitation both by month and as total sum, influenced the maize growing and development and also the yield of grain is not so clear. To clarify the importance of precipitation, we calculate the hydrothermal coefficient as suggested by Selyaninov (1928) for the main stages of the reproductive period of maize. For the location of Knezha this period begins relatively from June 20th and continues throughout the whole month of July. The calculation of the ratio of the average daily temperature sum and the precipitation through HTC characterizes in more detail the conditions in which this important reproductive stage of development of maize occurs (Qiao et al., 2025). It is evident from the HTC calculated values by year that they do not correspond to the rainfall amounts during the growing season. For example, the 2020 growing season has the least amount of rainfall (199.8 mm), but this during the reproductive stage together with the

temperature amount formed a coefficient of 0.73, while the 2021 growing season has the highest amount of rainfall (316.4 mm), the HTC coefficient was twice as low (0.36). The lowest hydrothermal coefficient for the reproductive stage was recorded in 2022 (0.15), despite the close precipitation amount to the average for the growing season (259.8 mm). This is mainly due to the scanty rainfall in July of 2022. The highest value of HTC was recorded in 2023 due to significant rainfall during the last ten days of June and the whole month of July.

Weather conditions affect the formation of grain yield of maize. The reported grain yields of maize hybrids and the values of the hydro-thermal coefficient are presented in Table 2.

Table 2. Grain yield from maize hybrids (kg/da) and HTC for the reproductive stage of the plants

	Hybrid \ Year	2020	2021	2022	2023	Average	% to Av.
FAO 400	Kneja 435	931.3	869.9	857.3	1007.9	916.6	99.5
	Kneja 461	911.4	901.6	898.5	992.4	925.9	100.5
	Average	921ş3	885.7	877.9	1000.1	921.3	
	LSD 0.05%	Ns	20.3	25.5	Ns	Ns	
FAO 500	Kneja 509	972.4	958.2	910.4	1003.2	961.1	96.9
	Kneja 561	1040.9	1004.2	977.1	1070.0	1023.1	103.1
	Average	1006.7	981.2	943.8	1036.6	992.1	
	LSD 0.05%	Ns	Ns	25.5	Ns	49.3	
	HTC*	0.73	0.36	0.15	1.26		

*Hydrothermal Coefficient

In each maturity group, two hybrids created at the Maize Institute - Knezha have been tested, one established in practice for decades (Kneja 435 and Kneja 509) and one new hybrid developed in recent years (Kneja 461 and Kneja 561).

The grain yields of all four hybrids varied according to the weather conditions during the growing seasons of each year. Tomov et al. (1995) found that in years of extreme drought, grain yield of maize was very low. On average, the lowest yields were obtained in 2022 (877.9 kg/da) for the FAO 400 maturity group and 943.8 kg/da for the FAO 500 maturity group, respectively. The grain yields obtained in the experimental years 2020 and 2021 were relatively similar, while the highest yields were obtained in 2023. This variation over the study period is consistent with the HTC for the maize generative period by year. Comparing the yields of the hybrids in the FAO 400 maturity group, there was evidence of an excess of yields of Kneja 461 in the years with the most pronounced drought during the generative period, namely in 2021 and especially in 2022, with HTC of 0.36 and 0.15, respectively. On average over the period, no differences were found between the yields of the Kneja 435 and Kneja 461 hybrids due to similar yields in 2020 and 2023.

For the FAO 500 maturity group hybrids, it can be seen that hybrid Kneja 561 has the highest grain yields in all years, but only in 2022 the HTC has a value of 0.15. The difference for 2022 and the average over the study period is statistically significant.

Considering the importance of maize and its cultivation potential worldwide and in Bulgaria, this crop's yield sustainability was studied in previous research. Genotype × environment interaction (GEI) is responsible for genotype sustainability characteristics. One of the usual exacting effects in plant breeding development is to dissect the genotype × environment (GE) interaction correctly. Genotype × environment interaction adjusts the reasonable grain yield of genotypes in diverse environments and makes it hard to select the better ones (Miah et al., 2016).

The results of the Anova with replications analysis conducted for the hybrids in the FAO 400 maturity group showed that the year of cultivation factor with specific weather conditions influenced the results with a 71.8% part (Table 3). The maize hybrid factor was insignificant due to the close yields obtained in the first and last year, and the average over the period.

Our results are in agreement with those of Romay et al. (2010). Similar to ours were the findings of Sárvári and Pepó (2014).

Considering only the yields obtained in 2021 and 2022, which had a certain drought during the generative period of maize (HTC - 0.36 and 0.15), the influence of the hybrid factor was found to be significant and that of the year insignificant (Table 4). This leads us to conclude that the new hybrid Kneja 461 in years with pronounced drought during the generative period has increased resistance, and in such conditions was less affected by adverse abiotic factors and provides stable yield.

Wilhelm et al. (2004) for long time period also suggested insufficient summer precipitation were major constraints for maximum maize yield. The same is for the studies of Malone et al. (2009).

Table 3 Anova with replications for the Kneja 435 and Kneja 461 hybrids for the period 2020-2023

Source of Variation	SS	df	MS	F	P-value	F crit	Factor	%
Year	75014	3	25004	26.3	9,1E-08	3,00	Year	71.8
Hybrid	707	1	707	0.7	0.397	4.26	Hybrid	0.7
Interaction	5995	3	1998	2.1	0.126	3.00		
Within	22778	24	949				Error	27.5
Total	104495	31						

Table 4 Anova with replications for the hybrids Kneja 435 and Kneja 461 for 2021 and 2022

Source of Variation	SS	df	MS	F	P-value	F crit	Factor	%
Hybrid	5340.0	1	5339	29.8	0.001	4.74	Hybrid	68.4
Year	240.4	1	240	1.3	0.269	4.74	Year	3.1
Interaction	87.0	1	86.5	0.4	0.500	4.74		
Within	2145.6	12	178.8				Error	28.6
Total	240.4	1	240.4	1.3	0.269	4.747		

The results of the ANOVA with replications analysis carried out for the hybrids in the FAO 500 maturity group showed that the year of cultivation factor with the specific weather conditions and the hybrid affected the results, albeit by a lower percentage, 37.7 and 31.2%, respectively (Table 5).

Table 5 Anova with replications for the Kneja 509 and Kneja 561 hybrids for the period 2020-2023

Source of Variation	SS	df	MS	F	P-value	F crit	Factor	%
Hybrid	37172.5	3	12390.8	9.88	0.00198	3.009	Year	37.7
Year	30740.7	1	30740.7	24.51	4,7E-05	4.260	Hybrid	31.2
Interaction	686.5081	3	228.8	0.18	0.907	3.009		
Within	30093.8	24	1253.9				Error	31.2
Total	98693.5	31						

When comparing the yields formed in years with the most pronounced drought during the reproductive period of maize (2021 and 2022), applying Anova with replications, a significant influence of the hybrid factor (40.0%) and a lesser influence of the year was found, as the calculated HTC had similar values of 0.36 and 0.15, respectively (Table 6).

Table 6 Anova with replications for the Kneja 509 and Kneja 561 hybrids for 2021 and 2022

Source of Variation	SS	df	MS	F	P-value	F crit	Factor	%
Hybrid	12702.9	1.0	12702.9	11.75	0.005	4.747	Hybid	40.0
Year	5612.9	1.0	5612.9	5.19	0.042	4.747	Year	17.7
Interaction	429.5	1.0	429.5	0.39	0.540	4.747		
Within	12968.1	12.0	1080.7				Error	42.2
Total	31713.4	15.0						

From the obtained results of this analysis, we can conclude that the newly developed hybrid Kneja 561 was less affected by the occurrence of drought during the reproductive period compared to Kneja 509 and consequently the obtained yields are more stable in years with such weather conditions.

Maize breeding in response to climate change aims to develop maize varieties that can reduce negative impacts on agriculture such as high temperatures, droughts. Kim and Lee (2023) suggested the factors for consideration in maize breeding in response to climate change. According to them these factors are high-temperature tolerance, drought tolerance, response to low precipitation. For this purpose should be developed maize varieties resistant to high temperatures; to select and improve maize varieties capable of maintaining both growth and productivity.

The obtained yields of the new hybrids Kneja 461 and Kneja 561 in years with pronounced droughts during the reproductive period of maize, which are superior to the established in practice Kneja 435 and Kneja 509, were the result of targeted selection in the direction of resistance to water deficit stress.

CONCLUSIONS

For the hybrids of maturity group FAO 400 in years with drought during the generative period of maize, the influence of the hybrid factor was found significant (68.4%) and insignificant of the year. The new hybrid Kneja 461 showed increased resistance, it was less affected by adverse abiotic factors and provided stable yield.

For hybrids of maturity group FAO 500 in years with pronounced drought during the generative period of maize, the influence of the hybrid factor was found (40.0%) and less influence of the year (17.7%). The newly developed hybrid Kneja 561 was less affected by drought compared to Kneja 509 and formed a more stable yield.

We can recommend since the environment very much influences genotypes, locations, and years, performance related to the stability of genotypes is a prerequisite and must carry out in different environments over different years in a given location.

ACKNOWLEDGEMENTS

This research work was carried out with the support of the Agricultural Academy - Sofia, Bulgaria.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

EVALUATION OF FOLIAR HERBICIDES FOR WEED CONTROL IN MAIZE (*Zea mays* L.).

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Abstract

In 2022 and 2023 a field experiment with the maize hybrid P 9610 was conducted. The trial was performed on the experimental field of the Agricultural University - Plovdiv, Bulgaria. The variants of the experiment were: 1. Untreated control; 2. Economic control; 3. Aminopielik 600 SL (1.20 l ha^{-1}); 4. Arigo WG (0.33 kg ha^{-1}); 5. Callam (0.40 kg ha^{-1}) 6. Starane Gold (1.20 l ha^{-1}). The herbicides were applied in the crop growth stage 3rd-5th leaf (BBCH 13-15). The weed infestation was represented by Chenopodium album L.; Amaranthus retroflexus L.; Xanthium strumarium L.; Abutilon theophrasti Medik., and Solanum nigrum L. The highest weed control against Ch. album, A. retroflexus, and A. theophrasti after the application of Callam was recorded, and against X. strumarium and S. nigrum after the application of Starane Gold. For the Economic control as well as for the variants treated with herbicides, the parameters ear diameter, ear length, number of seeds per ear cob, absolute seed mass, hectoliter seed mass and grain yield were higher and mathematically proven compared to the untreated control's results for these parameters.

Key words*: maize, weeds, herbicides, efficacy, biometry.*

INTRODUCTION

Providing sufficient raw materials to feed the growing population is one of the main challenges facing humanity. Agricultural crops are a major source of food products. Therefore, a large number of scientific studies are aimed at the successful and optimized production of agricultural crops (Panayotov et al., 2024; Mitkov, 2023; Rankova et al., 2023; Shopova, 2023; Dimtrova et al., 2019; Shopova & Cholakov, 2015; Dimitrova et al., 2014a; Dimitrova et al., 2014b; Shopova & Cholakov, 2014; Tityanov et al., 2010; Mitkov et al., 2009; Tityanov et al., 2009a).

Maize (*Zea mays* L.) is one of the most important cereal crops in the world (Ram et al., 2017). It is a vital source of carbohydrates and is used as animal feed, fuel, and raw materials for various industries, which determines its wide distribution area (Veeral & Abirami, 2021). In addition, maize has high adaptability and productive potential (Aldrich et al., 1975).

Weeds are one of the main factors hindering the maize cultivation. They compete with the crop for water, light, nutrients, and space, which leads to a decrease in yield and quality of the produced product (Tonev et al., 2019; Saleem et al., 2015; Arnold et al., 2013; Mundra et al., 2002). In addition, weeds can serve as hosts for the development of some parasitic plants, insects, and disease agents (Monteiro & Santos, 2022; Tonev et al., 2019).

The extent of yield reduction in maize depends on both the type and density of weeds and the duration of the competitive relationship between the crop and the weeds. Kumar et al. (2023a) found that prolonged weed development reduced the yield of *Z. mays* L. by up to 65.6%. Mukhtar et al. (2007) reported that if weeds are not destroyed, maize yield can decrease by 58% to 79%, which is accompanied by a 65% reduction in maize plant height. Other researchers also confirm the reduction in yield of *Z. mays* L. as a result of competition with weeds (Sharma & Rayamajhi, 2022; Gharde et al., 2018; Ehsas et al., 2016; Jagadish & Prashant, 2016; Kakade et al., 2016; Jat et al., 2012; Oerke & Dehne, 2004; Khan et al., 2003; Zhalnov & Raikov, 1996).

Sharma & Rayamajhi (2022) reported that the critical period for weed control in maize is between 4 and 7 weeks after sowing. Successful weed control during this period leads to the elimination of the harmful effect of weeds on crop yield. Fuadi & Wicaksono (2018) also reported that the first 30 days of maize growth are critical for weed control. The authors found

that if weeds are not controlled during this period, the yield of *Z. mays* L. can be reduced by 20% to 50%.

In both cultivated plants and corn, yield reduction, in addition to weed development, can also be caused by nutrient deficiency (Ivanov et al., 2021; Ivanov et al., 2019; Manolov & Neshev, 2017; Neshev & Manolov, 2016; Manolov et al., 2015; Neshev & Manolov, 2014; Neshev et al., 2014). Depending on the geographical latitude, agro-ecological conditions and crop cultivation technology, weeds of different species composition and density are widespread in maize fields. In Bulgaria, the following weeds are most often found with *Zea mays* L.: *Amaranthus retroflexus* L., *Datura stramonium* L., *Xanthium strumarium* L., *Solanum nigrum* L., *Chenopodium album*, *Abutilon theophrasti* L., *Sinapis arvensis* L., *Echinochloa crus gali* L., *Setaria glauca* L., *Sorghum halepense* L., *Convolvulus arvensis* L., *Cinodon dactilon* L. and *Cirsium arvense* L. (Yanev, 2023; Mitkov et al., 2019; Hristova et al., 2012; Kalinova et al., 2012; Mitkov et al., 2009).

Weed control in agricultural crops (Mitkov, 2024; Mitkov, 2021; Uzunova et al., 2015; Tityanov, et al., 2009b) including maize, is most often carried out through herbicide application (Bada et al., 2022; Mitkov, 2022; Goranovska & Kalinova, 2018; Goranovska et al., 2017; Sevov et al., 2015; Pannacci & Covarelli, 2009; Tonev et al., 2009b).

Dimitrova et al. (2013a) found that successful control of grass and broadleaf weeds could be achieved after soil application of Gardoprim plus gold 500 SK at a rate of 4.00 l ha^{-1}, Lumax 538 SK at a rate of 4.00 l ha^{-1} and Wing at a rate of 4.00 l ha^{-1}. Mitkov et al. (2018) recommended the application of Merlin Duo at rates of 1.00 l ha^{-1} to 2.00 l ha^{-1} after sowing before maize emergence for the control of *Abuthilon theophrasti* L. and *Solanum nigrum* L.

Very good efficacy against *Amaranthus retroflexus* L., *Setaria viridis* (L.) Beauv., *Sinapis arvensis* L. and *Solanum nigrum* L. was achieved after treatment with foramsulfuron at a rate of 20.3 g ai ha^{-1}. At a higher dose of 20 to 50 g ai ha^{-1}, the herbicide also provides very good control against *Abutilon theophrasti* Medik., *Chenopodium album* L. and *Echinochloa crus-galli* (L.) Beauv. (Pannacci, 2016).

Kalinova et al. (2000) reported that a system application of Stomp 33 EK (3.00 l ha^{-1}) and Mistral 4 SK (1.30 l ha^{-1}) in maize controlled *Sorghum halepense* L., *Convolvulus arvensis* L., *Echinochloa crus gali* L., *Chenopodium album* L., *Amaranthus retroflexus* L. and *Abutilon theophrasti* L.

In the presence of mixed infestations of cereal and broadleaf weeds, Kierzek et al. (2012) recommend soil application of a tank mix of s-metolachlor + terbuthylazine + mesotrione and vegetative use of nicosulfuron with the adjuvant Atpolan Bio 80 SL.

A large number of scientists have established the biological efficacy of atrazine in combination with other herbicides for weed control in maize (Acharya et al., 2022; Bottcher et al., 2022; Burhanuddin Wiqar et al., 2022; Choudhary et al., 2022; Jha et al., 2022; Khanna et al., 2022; Pinsupa et al., 2022; Wasnik et al., 2022). Soil application of atrazine followed by vegetative treatment with tembotrione has been found to successfully control weeds in maize (Arunkumar et al., 2019). Bada et al. (2022) also reported effective weed control using a system involving soil application of atrazine followed by foliar treatment with tembotrione or topramezone. Kurniadie et al. (2023) recommended the use of a herbicide mixture of atrazine 265 g/l and nicosulfuron 35 g/l at doses of 1.0 l/ha to 3 l/ha for successful control of *Bidens pilosa, Ageratum conyzoides, Synedrella nodiflora, Richardia brasiliensis, Eleusine indica* and *Digitaria ciliaris*.

According to Damalas et al. (2018), when weeding maize with *Xanthium strumarium, Amaranthus retroflexus, Datura stramonium* and *Chenopodium album* very good control can be achieved after applying tembotrione at 100 g ai ha^{-1} and tembotrione at 100 g ai ha^{-1} in combination with rimsulfuron at 10 g ai ha^{-1}, nicosulfuron at 40 g ai ha^{-1} and foramsulfuron at 60 g ai ha^{-1}.

The aim of the present study was to evaluate foliar herbicides for weed control in maize (*Zea mays* L.).

MATERIALS AND METHODS

In 2022 and 2023, a field experiment with maize, hybrid P 9610, was conducted in the experimental field of the Department of Agriculture and Herbology at the Agricultural University - Plovdiv. The experiment was conducted using the randomized block method (Dimova & Marinkov, 1999) in 4 replications, with a plot size of 20 m².

The experiment variants include: 1. Untreated control - without digging and herbicides; 2. Economic control - earthed-up, without herbicides; 3. Aminopielik 600 SL (2.4 D amine salt 600 g/l) at a rate of 1.20 l ha^{-1}; 4. Arigo WG (Nicosulfuron 120 g/kg + Mesotrione 360 g/kg + Rimsulfuron 30 g/kg) at a rate of 0.33 kg ha^{-1}; 5. Callam (Dicamba 600 g/kg + Tritosulfuron 125 g/kg) at a rate of 0.40 kg ha^{-1}; 6. Starane Gold (Florasulam 1 g/l + Fluroxypyr 100 g/l) at a rate of 1.20 l ha^{-1}. The herbicides were applied in the phenophase 3rd-5th leaf of the crop with a backpack electric sprayer SOLO (model 417), with a working solution consumption of 210 l ha^{-1}.

The corn was grown after a corn predecessor (monoculture) under non-irrigated conditions. The experimental area was fertilized with 250 kg ha^{-1} combined N:P:K (15:15:15) fertilizer, after which the soil was deeply plowed. Before sowing the crop, a 15 cm disking was performed followed by two cultivations at a depth of 8 cm. The corn was sown at the optimal time for the region, with a density of 65,000 plants per hectare. Spring fertilization with 25 kg/da NH_4NO_3 was also carried out.

During the two years of the study, weeding in the field was represented by the following weed species: *Chenopodium album* L.; *Amaranthus retroflexus* L.; *Xanthium strumarium* L.; *Abutilon theophrasti* Medik. and *Solanum nigrum* L. The efficacy of the herbicides was assessed using the 10-point visual scale of EWRS on the 14th, 28th and 56th days after treatment. The selectivity of the herbicides was assessed on the 7th, 14th and 28th days after application. For this purpose, the 9-point scale of EWRS was used, where at a score of 1 - there is no damage to the crop, and at a score of 9 there is complete death of the crop (Zhelyazkov et al. 2017).

The following corn parameters were recorded and analyzed - ear diameter (cm), ear length (cm), number of seeds per ear cob, absolute seed mass (g), hectoliter seed mass (kg) and grain yield (t ha^{-1}). Duncan's method was used for statistical data processing with the SPSS 19 program (Duncan, 1955). Differences were considered significant at $p<0.05$.

RESULTS AND DISCUSSIONS

The results of the studies show that, with the exception of sow thistle, the biological efficacy of the applied herbicides increased from the 14th to the 56th day for the remaining weeds present in the experiment (Tables 1 to 6). On average, for the period of the 14th day after application of the studied herbicides, the highest efficacy against *Chenopodium album* L. was recorded for Aminopielik 600 SL at a rate of 1.20 l ha^{-1} - 60%, followed by Callam at a dose of 0.40 kg ha^{-1} - 57.5%. The weakest control against the weed was recorded for Starane Gold (1.20 l ha^{-1}) - 25%. On the 28th day, the highest efficacy was recorded after treatment with Callam - 75%. Again, the control after the application of Starane Gold was unsatisfactory – 35%. During the last reporting date, very good efficacy against *Ch. album* was recorded for Callam – 90%. The application of Aminopielik 600 SL and Arigo WG provided good control against the weed – 80%. After the application of 2, 4-D sodium salt 80% WP at a dose of 1,250 g ha^{-1} in maize Kumar et al. (2023b) found almost the same efficacy - 77.3% in the control of *Chenopodium album* (L.), *Physalis minima* Roem. & Schult, *Melilotus indicus* (L.) and *Cichorium intybus*. Of all the herbicides studied in the experiment, the lowest efficacy against *Ch. album* L. was reported for Starane Gold - 45% (Table 1).

Of the weeds present in the experiment, *Amaranthus retroflexus* L. is the most sensitive to the applied herbicides. On the first reporting date, the highest control was after the application of Aminopielik 600 SL - 70%, followed by Arigo WG and Callam - 65%. On the second reporting, an increase in efficacy was observed after the application of the herbicides, with the highest being in the variant with Arigo WG - 85%.

On the 56th day, very good control against *Am. retroflexus* L. - 95% was observed with Arigo WG and Callam. The other two herbicides also successfully controlled the weed, with the efficacy for Aminopielik 600 SL being 90%, and for Starane Gold - 87.5% (Table 2).

Table 1. Efficacy (%) of the studied herbicides against *Chenopodium album* L., day after treatment (DAT)

Variants	2022			2023			Average		
	14 DAT	28 DAT	56 DAT	14 DAT	28 DAT	56 DAT	14 DAT	28 DAT	56 DAT
1. Untreated control	0	0	0	0	0	0	0	0	0
2. Economic control	100	100	100	100	100	100	100	100	100
3. Aminopielik 600 SL (1.20 l ha^{-1})	55.0	70.0	75.0	65.0	70.0	85.0	60.0	70.0	80.0
4. Arigo WG (0.33 kg ha^{-1})	45.0	65.0	75.0	55.0	75.0	85.0	50.0	70.0	80.0
5. Callam (0.40 kg ha^{-1})	55.0	70.0	90.0	60.0	80.0	90.0	57.5	75.0	90.0
6. Starane Gold (1.20 l ha^{-1})	30.0	40.0	50.0	20.0	30.0	40.0	25.0	35.0	45.0

Table 2. Efficacy (%) of the studied herbicides against *Amaranthus retroflexus* L., day after treatment (DAT)

Variants	2022			2023			Average		
	14 DAT	28 DAT	56 DAT	14 DAT	28 DAT	56 DAT	14 DAT	28 DAT	56 DAT
1. Untreated control	0	0	0	0	0	0	0	0	0
2. Economic control	100	100	100	100	100	100	100	100	100
3. Aminopielik 600 SL (1.20 l ha^{-1})	70.0	75.0	85.0	70.0	85.0	95.0	70.0	80.0	90.0
4. Arigo WG (0.33 kg ha^{-1})	60.0	80.0	90.0	70.0	90.0	100	65.0	85.0	95.0
5. Callam (0.40 kg ha^{-1})	60.0	75.0	90.0	70.0	85.0	100	65.0	80.0	95.0
6. Starane Gold (1.20 l ha^{-1})	60.0	75.0	85.0	60.0	85.0	90.0	60.0	80.0	87.5

Table 3. Efficacy (%) of the studied herbicides against *Xanthium strumarium* L., day after treatment (DAT)

Variants	2022			2023			Average		
	14 DAT	28 DAT	56 DAT	14 DAT	28 DAT	56 DAT	14 DAT	28 DAT	56 DAT
1. Untreated control	0	0	0	0	0	0	0	0	0
2. Economic control	100	100	100	100	100	100	100	100	100
3. Aminopielik 600 SL (1.20 l ha^{-1})	55.0	45.0	35.0	65.0	55.0	45.0	60.0	50.0	40.0
4. Arigo WG (0.33 kg ha^{-1})	65.0	55.0	35.0	70.0	65.0	45.0	67.5	60.0	40.0
5. Callam (0.40 kg ha^{-1})	50.0	65.0	75.0	60.0	70.0	85.0	55.0	75.0	80.0
6. Starane Gold (1.20 l ha^{-1})	45.0	65.0	80.0	55.0	75.0	90.0	50.0	70.0	85.0

Table 4. Efficacy (%) of the studied herbicides against *Abutilon theophrasti* Medik. L., day after treatment (DAT)

Variants	2022			2023			Average		
	14 DAT	28 DAT	56 DAT	14 DAT	28 DAT	56 DAT	14 DAT	28 DAT	56 DAT
1. Untreated control	0	0	0	0	0	0	0	0	0
2. Economic control	100	100	100	100	100	100	100	100	100
3. Aminopielik 600 SL (1.20 l ha^{-1})	80.0	90.0	95.0	70.0	80.0	95.0	75.0	85.0	95.0
4. Arigo WG (0.33 kg ha^{-1})	50.0	75.0	80.0	55.0	85.0	90.0	52.5	80.0	85.0
5. Callam (0.40 kg ha^{-1})	50.0	70.0	95.0	60.0	80.0	95.0	55.0	75.0	95.0
6. Starane Gold (1.20 l ha^{-1})	40.0	55.0	80.0	40.0	65.0	80.0	40.0	60.0	80.0

Table 5. Efficacy (%) of the studied herbicides against *Solanum nigrum* L., day after treatment (DAT)

Variants	2022			2023			Average		
	14 DAT	28 DAT	56 DAT	14 DAT	28 DAT	56 DAT	14 DAT	28 DAT	56 DAT
1. Untreated control	0	0	0	0	0	0	0	0	0
2. Economic control	100	100	100	100	100	100	100	100	100
3. Aminopielik 600 SL (1.20 l ha^{-1})	50.0	70.0	80.0	60.0	75.0	80.0	55.0	75.0	80.0
4. Arigo WG (0.33 kg ha^{-1})	60.0	75.0	90.0	65.0	80.0	90.0	62.5	77.5	90.0
5. Callam (0.40 kg ha^{-1})	45.0	65.0	70.0	55.0	70.0	80.0	50.0	67.5	75.0
6. Starane Gold (1.20 l ha^{-1})	60.0	75.0	85.0	65.0	85.0	95.0	62.5	80.0	90.0

One of the difficult weeds to control in the experiment was *Xanthium strumarium* L. It was found that when using Arigo WG and Aminopielik 600 SL, the highest efficacy was on the 14th day, 67.5% and 60%, respectively. During the remaining two reports, the control

with these two preparations decreased, with the lowest being on the 56th day - 40% for Arigo WG and Aminopielik 600 SL. The opposite was observed with the remaining two herbicides. The highest efficacy after the application of Starane Gold and Callam was reported on the 56th day after treatment. Of the herbicides studied, the best control against *X. strumarium* was provided by Starane Gold, where on the third reporting date the average for the period was 85% (Table 3).

The highest efficacy against *Abutilon theophrasti* Medik. on the 14th day was registered after the application of Aminopielik 600 SL - 75%. In the other herbicide variants, the control was significantly lower, ranging from 40% to 55%. On the 28th day, an increase in efficacy was reported, with Aminopielik 600 SL reaching 85% and Arigo WG reaching 80%. On the 56th day, the best control against *A. theophrasti* Medik. - 95% was reported after the use of Aminopielik 600 SL and Callam. Of all the herbicides studied, the weakest control was in the variant with Starane Gold - 80%. (Table 4).

The results related to the biological efficacy of the applied herbicides against *Solanum nigrum* L. show that on the 14th day the best control was with Arigo WG and Starane Gold - 62.5%. The application of Aminopielik 600 SL and Callam provided 55% and 50% efficacy against the weed, respectively. On the 28th day the best control against *S. nigrum* L. was reported with Starane Gold – 80%. Similar efficacy was also found in the variants with Arigo WG and Aminopielik 600 SL, 77.5% and 75%, respectively. Of all the herbicides studied on the 56th day, on average for the period, the best control against the weed was registered after the use of Starane Gold - 90% and Arigo WG - 90%. On the last reporting date, Aminopielik 600 SL provided 80%, and Callam - 75% efficacy against *S. nigrum* L. (Table 5).

In addition to assessing the control of the applied herbicides against the available weeds during the two experimental years, the selectivity of the products towards maize, hybrid P 9610, was also monitored. Under the conditions of the experiment, on the 7th, 14th and 28th day after the application of the herbicides, no visible symptoms of phytotoxicity were detected - score 1 on the EWRS scale.

During the experiment, the productivity of the maize hybrid P 9610 was monitored, both in the variants treated with herbicides, and in the Untreated and Economic controls. It was found that the largest cob diameter in both years was recorded in the Economic control and Callam (0.40 kg ha^{-1}), on average 3.71 cm and 3.64 cm. It is correct to note that there is no statistically proven difference between these two variants. It has been proven that there is no difference between Starane Gold (1.20 l ha^{-1}), Arigo WG (0.33 kg ha^{-1}) and Aminopielik 600 SL (1.20 l ha^{-1}) in terms of the studied indicator. The lowest ear diameter of all variants was found in the untreated control, as in 2022 it was 2.37 cm, and in 2023 it was 2.21 cm. The results were mathematically proven according to Duncan's multiple range test ($p < 0.05$) (Table 6).

Table 6. Ear diameter, cm

Variants	2022	2023	Average
1. Untreated control	2.37 c	2.21 c	2.29
2. Economic control	3.75 a	3.67 a	3.71
3. Aminopielik 600 SL (1.20 l ha^{-1})	3.23 b	3.20 b	3.22
4. Arigo WG (0.33 kg ha^{-1})	3.32 b	3.19 b	3.26
5. Callam (0.40 kg ha^{-1})	3.66 a	3.61 a	3.64
6. Starane Gold (1.20 l ha^{-1})	3.35 b	3.23 b	3.29

Variants with different letters are with proved difference according to Duncan's multiple range test ($p < 0.05$).

Under the conditions of the experiment, the longest ears were recorded in the Economic control and Callam (0.40 kg ha^{-1}). In 2022, in the Economic control, the ear length was 14.90 cm, and in 2023 it was 14.35 cm. Close to these values were recorded after the application of Callam as well, where in 2022 the ear length was 14.55 cm, and in 2023 it was 14.10 cm. No mathematically proven difference was found between variants 2 and 5. The results of the Duncan test showed that in 2022 there was no proven difference between Callam (14.55 cm), Starane Gold (13.65 cm) and Arigo WG (13.65 cm), but there was a significant difference between Aminopielik 600 SL (13.25 cm) and Callam (14.55 cm). In 2023, it was found that the ear length in the Economic control and Callam was proven to be higher than the variants with Arigo WG (12.75 cm) and Aminopielik 600 SL (12.25 cm). Again, the shortest cob length was in the Untreated control plants – an average of 10.58 cm where we do not use herbicides and hoeing (Table 7).

Table 7. Ear lenght, cm

Variants	2022	2023	Average
1. Untreated control	10.85 d	10.30 d	10.58
2. Economic control	14.90 a	14.35 a	14.63
3. Aminopielik 600 SL (1.20 l ha^{-1})	13.25 c	12.25 c	12.75
4. Arigo WG (0.33 kg ha^{-1})	13.65 bc	12.75 c	13.20
5. Callam (0.40 kg ha^{-1})	14.55 ab	14.10 ab	14.33
6. Starane Gold (1.20 l ha^{-1})	13.65 bc	13.00 bc	13.33

Variants with different letters are with proved difference according to Duncan's multiple range test ($p < 0.05$).

Regarding the indicator number of seeds per mazie ear, the lowest values were proven in the untreated control, where in 2022 they were 215.00, and in 2023 they were 207.70.

It has been statistically proven that the highesrt number of seeds in the Economic control and Callam was recorded.

In variants 2 and 5, the number of seeds in 2022 was 359.75 and 357.20, respectively, and in 2023 it was 341.40 and 338.75. No mathematically proven difference was fund between the Economic control and Callam (Table 8).

Table 8. Number of seeds per mzize ear

Variants	2022	2023	Average
1. Untreated control	215.00 c	207.70 d	211.35
2. Economic control	359.75 a	341.40 a	350.58
3. Aminopielik 600 SL (1.20 l ha^{-1})	322.15 b	303.50 c	312.83
4. Arigo WG (0.33 kg ha^{-1})	336.35 ab	320.20 bc	328.28
5. Callam (0.40 kg ha^{-1})	357.20 a	338.75 ab	347.98
6. Starane Gold (1.20 l ha^{-1})	330.50 b	319.20 bc	324.85

Variants with different letters are with proved difference according to Duncan's multiple range test ($p < 0.05$).

The results related to the absolute seeds mass showed that it is proven to be the highest in the Economic control and Callam - an average of 265.68 g and 261.48 g. There was no mathematically proven difference between these two variants.

The highest values of the indicator in variants 2 and 5 correspond to the highest results in terms of weed control. Other authors also found higher values of the absolute mass of seeds after successful weed control (Bastegan et al., 2022; Fang et al., 2022).

The lowest absolute seed mass in both years was found in the Untreated control - an average of 183.55 g. It is correct to note that proven higher values of this indicator than in the untreated control were recorded after the application of Arigo WG, Starane Gold and Aminopielik 600 SL (Table 9).

Table 9. Absolute seed mass, g

Variants	2022	2023	Average
1. Untreated control	189.00 c	178.10 c	183.55
2. Economic control	271.20 a	260.15 a	265.68
3. Aminopielik 600 SL (1.20 l ha^{-1})	226.60 b	216.60 b	221.60
4. Arigo WG (0.33 kg ha^{-1})	243.35 b	226.90 b	235.13
5. Callam (0.40 kg ha^{-1})	266.10 a	256.85 a	261.48
6. Starane Gold (1.20 l ha^{-1})	232.25 b	220.75 b	226.50

Variants with different letters are with proved difference according to Duncan's multiple range test ($p < 0.05$).

The highest values for the hectoliter seed mass were variants 2 and 5. In the Economic control in 2022, the hectoliter mass was 75.25 kg, and in 2023 it was 71.15 kg. Almost the same values of the indicator were recorded after the application of Callam, where in 2022 it was 75.00 kg, and in 2023 it was 69.50 kg. There was no proven difference between these two variants according to Duncan's multiple range. For Starane Gold, Arigo WG and Aminopielik 600 SL, during the two years of study, the values of the indicator varied from 64.15 kg to 60.40 kg, and there is no mathematically proven difference between them. Of all the variants in the trial, the lowest hectoliter mass in the Untreated control was recorded, where in 2022 it was 56.35 kg, and in 2023 - 53.15 kg (Table 10).

Table 10. Hectoliter seed mass, kg

Variants	2022	2023	Average
1. Untreated control	56.35 c	53.15 c	54.75
2. Economic control	75.25 a	71.15 a	73.20
3. Aminopielik 600 SL (1.20 l ha^{-1})	62.60 b	60.40 b	61.50
4. Arigo WG (0.33 kg ha^{-1})	63.15 b	62.90 b	63.03
5. Callam (0.40 kg ha^{-1})	75.00 a	69.50 a	72.25
6. Starane Gold (1.20 l ha^{-1})	64.15 b	63.60 b	63.88

Variants with different letters are with proved difference according to Duncan's multiple range test ($p < 0.05$).

Under the conditions of the study, the lowest proven maize grain yield, hybrid P 9610, was recorded in the Untreated control, where no measures were no weed management was done. The yield for the Untreated control in 2022 was 3.17 t ha^{-1}, and in 2023 it was 2.94 t ha^{-1} (Table 11). Many scientists have proven that the high weed infestation leads to yield decrease, the extent of which depends on the type and density of the weeds (Manilov & Manhart, 2024; Mitkov, 2024; Choudhary et al., 2022; Mitkov, 2022; Wiqar et al., 2022; Mitkov 2020; Tursun et al., 2016; Skrzypczak et al., 2011; Tonev et., al., 2010; Tonev et al., 2009a; Walia et al., 2005).

From all studied variants of the experiment, the highest maize yield in the Economic control and after the application of Callam was recorded. As a result of weed control in 2022, the yield in the Economic control was 6.47 t ha^{-1}, and in 2023 it was 6.25 t ha^{-1}. Other authors also reported that when weeds are controlled by hoeing, better conditions are created for the growth and development of corn (Kumar et al., 2017; Sarma et al., 2010). In second place in terms of yield was the variant with Callam, with 6.41 t ha^{-1} in 2022 and 6.19 t ha^{-1} in 2023. There was no proven difference between the two variants according to Duncan's multiple range test ($p < 0.05$). It is worth noting that the herbicide treatments with Arigo WG, Starane Gold, and Aminopielik 600 SL also proved to increase the mazie yields when compared to that obtained from the Untreated control. The obtained results confirm the positive correlation between the efficacy of herbicides against weeds and the yields.

Tabele 11. Maize grain seed yield (t ha^{-1})

Variants	2022	2023	Average
1. Untreated control	3.17 c	2.94 c	3.06
2. Economic control	6.47 a	6.25 a	6.36
3. Aminopielik 600 SL (1.20 l ha^{-1})	6.02 b	5.80 b	5.91
4. Arigo WG (0.33 kg ha^{-1})	6.14 b	5.89 b	6.02
5. Callam (0.40 kg ha^{-1})	6.41 a	6.19 a	6.30
6. Starane Gold (1.20 l ha^{-1})	6.05 b	5.86 b	5.96

Variants with different letters are with proved difference according to Duncan's multiple range test ($p < 0.05$).

CONCLUSIONS

During the experimental period, the highest herbicidal efficacy against *Chenopodium album* L., *Amaranthus retroflexus* L. and *Abutilon theophrasti* Medik. L. was found on the 56th day after the application of Callam at a rate of 0.40 kg ha^{-1}, 90%, 95% and 95%, respectively. Of the herbicides studied treatment with Starane Gold at a dose of 1.20 l ha^{-1} provided the best control against *Xanthium strumarium* L. and *Solanum nigrum* L., with 85% and 90%, respectively, on the 56th day after the treatment.

Under the conditions of the experiment, after the application of the studied herbicides, no visible symptoms of phytotoxicity were detected for the maize hybrid P 9610 grown in the study.

Ear diameter, ear length, number of seeds per ear, absolute seed mass, hectoliter seed mass and grain yield were proven to be highest for the Economic control and after the use of Callam.

ACKNOWLEDGEMENTS

The research was financially supported by Project 17-12 at the Center of Research, Technology Transfer and Protection of Intellectual Property Rights at the Agricultural University of Plovdiv, Bulgaria.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

RELATIONSHIPS BETWEEN MORPHOMETRIC AND QUALITY PARAMETERS IN WHEAT GRAINS

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Abstract

Wheat is a staple food grain that plays a vital role in daily diets worldwide. Therefore, even minor changes in wheat production and quality hold significant importance globally, particularly in today`s context, where food crises are a pressing concern. While the effects of various environmental factors on wheat yield and quality have been extensively studied, research on the variation in quality traits of seeds from wheat plants exposed to the same environmental conditions remains limited. In this study, the quality traits and amino acid contents of wheat seeds grown under identical conditions were comparatively analyzed based on their size. The results showed that protein content, sedimentation value, and gluten levels were highest in medium-sized seeds, whereas starch content was found to be higher in large seeds. Moreover, essential amino acids crucial for human health were observed at the highest levels in smaller seeds of the same variety.

Key words*: wheat, morphometric traits, quality parameters, seed size.*

INTRODUCTION

Among cereal crops, wheat has a significantly larger cultivation area and higher production volume compared to others, as a result of being a fundamental source of calories (Reynolds and Braun, 2022; FAO, 2024).

Wheat production and the quality of the harvested product depend on various environmental factors (Woggoner, 1969). Numerous studies have demonstrated the impact of environmental factors such as precipitation (Tatar et al., 2020), air temperature (Mahdavi et al., 2022), and soil nutrient content (Fangmeier et al., 1999) on wheat yield and quality. Although the effects of environmental factors and genetic differences on grain characteristics and quality have been extensively studied, research on the variation within the same seed lot remains limited. In this context, morphometric differences like grain size and weight distribution are hypothesized to play a crucial role in determining the resulting flour properties and nutritional aspects within the same harvest (Aydoğan et al., 2014).
Wheat grain morphology is not only crucial for yield attributes but also influences grain quality parameters such as nutritional composition and milling performance. In fact, grain size and shape have been shown to directly affect the end-use quality and market value of wheat (Gegas et al., 2010). Recognizing these influences provides essential context for linking physical grain characteristics with quality metrics in modern cultivars, underscoring the importance of studying their interrelationships.
Hence, this study aims to reveal the differences in composition and quality of bread wheat seeds obtained from the same harvest, but classified by their grain size, shedding light on the importance of in-lot variability and its implications for wheat utilization.

MATERIALS AND METHODS

The experiment was conducted at the Department of Field Crops, Faculty of Agriculture, Ege University. As plant material, a total of 500 g of bread wheat (*Triticum aestivum* L.) seeds obtained from the same field and the same growing season were used. From these seeds, 100 seeds were sampled in four replications, and the thousand grain weight (TGW) was determined. The seeds with

calculated TGW values (500 g) were classified into three categories based on their weight: small, medium, and large (Table 1).

Table 1. The ranges used in the classification process of bread wheat (*Triticum aestivum* L.) seeds (500 g) based on their TGW into three categories are as follows

Classification:	Ranges Based on Grain Weights
Small grains	<TGW x 0.95
Medium grains	TGW x 0.95 ~ TGW x 1.05
Large grains	>TGW x 1.05

The classification process was conducted using a precision balance, with each seed weighed individually (Figure 1).

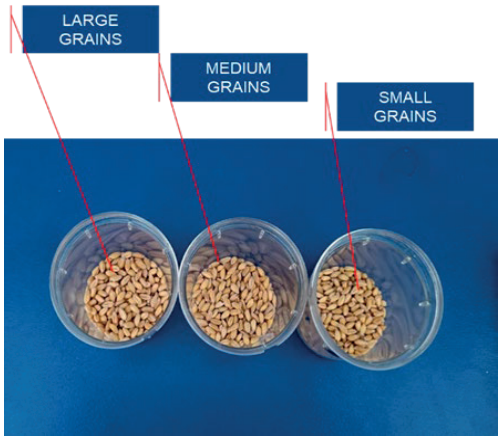


Figure 1. Image of the classification process of bread wheat (*Triticum aestivum* L.) seeds based on their TGW

After the separation process, TGW measurements were repeated for each group (small, medium, and large grains). In the obtained three seed groups, the following values were determined using an NIR spectrometer:
1) quality parameters: Wet gluten (%), Protein (%), Sedimentation (mL), Falling number (s), Hectolitre (g/L), Fat (%), Fiber (%);
2) amino acids: Leucine, Phenylalanine, Isoleucine, Egg + Cysteine, Arginine, Valine.

RESULTS AND DISCUSSIONS

Thousand Grain Weight (TGW)

The TGW values of *Triticum aestivum* L. seeds classified as small, medium, and large are presented in Figure 2. The TGW values of the classified seeds were found to be 53.3 g for large grains, 44.1 g for medium grains, and 37.4 g for small grains.

The obtained TGW values indicate that the classification performed by individually weighing the seeds (Table 1) is reflected in the TGW values of each group. As a result of the classification, the TGW of medium-sized seeds was 17.9% higher than that of small seeds, while large seeds had a 42.5% higher TGW compared to small seeds. These values and the observed differences between the groups were considered sufficient for comparing quality traits and grain composition data.

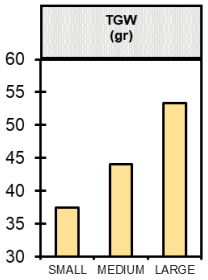


Figure 2. Thousand Grain Weight (TGW) values of *Triticum aestivum* L. seeds classified as small, medium, and large

Grain Quality Characteristics

The values of wet gluten (%), protein content (%), sedimentation (mL), and falling number (s) for *Triticum aestivum* L. seeds classified as small, medium, and large are presented in Figure 3.

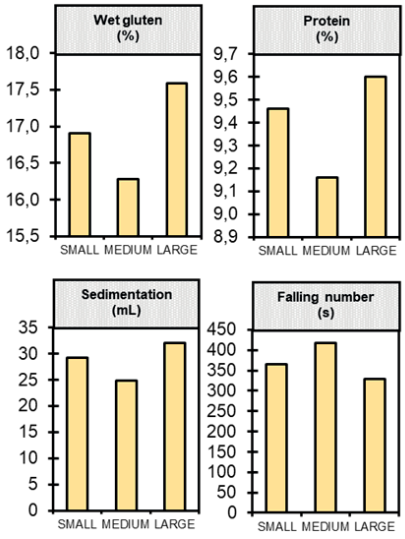


Figure 3. Wet gluten (%), protein content (%), sedimentation (mL), and falling number (s) values of *Triticum aestivum* L. seeds classified as small, medium, and large

Several studies have demonstrated that as grain weight decreases in wheat, there is a decline in protein content, gluten, and sedimentation values (Mahdavi et al., 2022). For instance, Aydoğan et al. (2014) reported in a multi-cultivar study that as grain size decreased, the protein content and dry gluten content of wheat increased, whereas larger grains showed higher sedimentation values. This inverse relationship between grain size and protein concentration is also supported by other research: Wang and Fu (2020) observed that when kernel size decreased in durum wheat, the protein content rose significantly (from 14.1% up to 18.6% in the smallest kernels). Generally, smaller or lighter kernels within a single variety have been associated with higher protein content and stronger gluten, whereas larger kernels often have slightly lower protein but can show good sedimentation values depending on protein quality (Aydoğan et al., 2014; Sahin et al., 2013). These trends are thought to arise because larger grains allocate more endosperm (mostly starch) during development, whereas smaller grains have a higher proportion of germ and aleurone layer which contain protein-rich tissues (Okur & Onel, 2024).

In the present study, when comparing the smallest and largest grain fractions, a similar pattern was observed in part: the large grains had lower protein percentage than the small grains (9.6% vs. an intermediate value of ~9.4% in small grains, based on the figures), consistent with the idea that very large kernels are more starchy. However, the medium-sized grains did not conform to the expected trend and actually exhibited the lowest protein, gluten, and sedimentation values among the three groups. This deviation is noteworthy. A possible explanation is related to the position of grains on the wheat spike. Within a single spike, grains that develop in different positions (top, middle, bottom) receive varying amounts of nutrients and assimilates. Boz et al. (2012) found that grain position significantly affects grain quality: grains from the middle of the spike were the largest, while protein content increased from the top to the bottom of the spike. It is also possible that the single variety used in this study has a unique allocation of resources among grains that does not strictly follow grain size. Genetic variation can modulate the relationship between grain size and quality traits. Thus, the lower protein and gluten in medium-sized grains (relative to both small and large) may reflect a complex interaction of developmental timing and genetic factors in this particular wheat lot. Further research isolating grains by their position on the spike, in addition to size, could clarify this anomaly.

In general, it is known that as the thousand grain weight (TGW) decreases in wheat, the hectoliter weight (HW) increases (Tatar et al., 2020). This is because smaller grains often pack more densely, filling a given volume more completely than larger grains. In our results, when only the small and large grain groups were considered, the expected inverse relationship between TGW and hectoliter weight was observed: the small grains had a relatively higher hectoliter weight than the very large grains (Figure 4), which is consistent with the literature. However, the medium-sized grains did not follow the same trend, showing a hectoliter weight that did not exceed that of the small grains. This could be related to their overall shape or density.

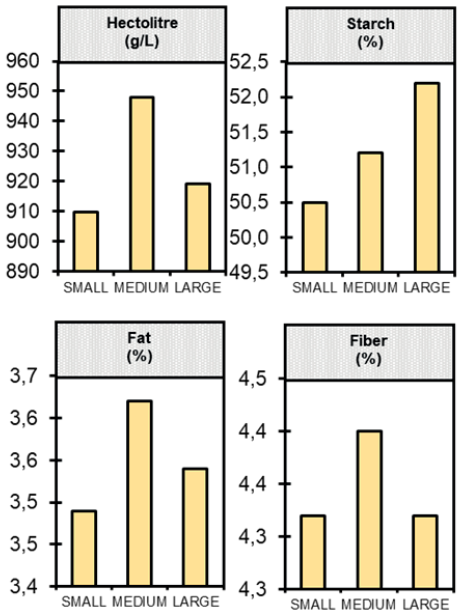


Figure 4. Hectoliter weight (g/L), starch (%), fat (%), and fiber (%) values of *Triticum aestivum* L. seeds classified as small, medium, and large

Starch content was found to increase proportionally with TGW. The large grains had the highest starch percentage, while smaller

grains had less starch. This can be explained by the fact that as grain size increases, the proportion of starchy endosperm in the seed also increases, diluting other components. The trends in the results align with this: large grains (heaviest) showed the highest starch content, consistent with their greater endosperm development. Conversely, small grains, having less endosperm, showed slightly lower starch percentages and higher relative protein. The literature supports this inverse relationship between starch and protein concentrations; as one increases, the other tends to decrease due to a dilution effect in the grain composition (Okur & Onel, 2024).

Erbaş Köse et al. (2013) observed that as TGW decreases in wheat grains, there is a corresponding decrease in fixed oil (fat) and fiber content. In other words, smaller grains were reported to have lower oil and fiber than larger grains in that study. However, in the present study, we did not observe a clear trend for oil (fat) and fiber content with respect to grain size (Figure 4). The fat and fiber percentages were relatively similar across small, medium, and large grains, without a statistically significant pattern. This discrepancy could be due to varietal differences or the narrow range of grain sizes in our classification. It is possible that the wheat variety examined does not exhibit significant variation in bran (outer layer) proportions between medium and small grains. Given that fiber is predominantly concentrated in the bran and oil in the germ, fractions with similar bran-to-germ ratios would likely have comparable fiber and fat contents (Erbaş Köse et al., 2013). Additionally, minor differences may not have been detected due to the sensitivity of NIR measurements. These findings suggest that compositional parameters do not uniformly vary with grain size; fiber and fat contents may remain relatively constant across different seed sizes within a single variety, whereas protein and starch contents exhibit more pronounced differences.

Grain Amino Acid Content

The amino acid contents of *Triticum aestivum* L. seeds classified as small, medium, and large based on thousand grain weight (TGW) are presented in Figure 5.

An analysis of amino acid composition revealed that the contents of leucine, phenylalanine, and isoleucine were slightly lower in medium-sized grains compared to both small and large grains. In contrast, the levels of Egg+Cys (methionine plus cysteine), arginine, and valine clearly decreased as grain size increased. In other words, for methionine+cysteine, arginine, and valine, the smallest grains had the highest concentrations, and the largest grains had the lowest, with medium grains in between. These trends suggest that smaller wheat seeds, despite having overall less mass and protein, contained a higher proportion of certain essential amino acids.

Esfandiari and Abdoli (2016) reported that, in addition to protein content, the biological properties of wheat grains, such as amino acid composition, are of great importance for nutrition. Similarly, Labanauskas et al. (1981) stated that the amino acid content of wheat varies significantly depending on environmental factors. The results indicate that not only environment (García del Moral et al., 2007) but also grain size (within the same environment) can influence the amino acid profile of the grain.

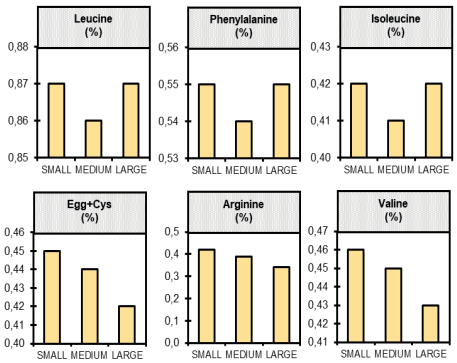


Figure 5. Leucine (%), phenylalanine (%), isoleucine (%), Egg+Cys (%), arginine (%), and valine (%) values of *Triticum aestivum* L. seeds classified as small, medium, and large

The obtained results clearly demonstrated that as TGW increases, the contents of Egg+Cys, arginine, and valine decrease. However, no significant change was observed in leucine, phenylalanine, and isoleucine levels in relation to TGW. One possible explanation for why smaller grains had higher percentages of certain amino acids is the distribution of protein types within the grain. Wheat grain proteins can be broadly categorized into gluten proteins

(prolamins, which are low in some essential amino acids) and non-gluten proteins (albumins and globulins, which often have more balanced amino acid profiles) (Adgunkar et al., 2023). Smaller grains might have a relatively larger contribution of proteins from the bran and germ (which are richer in lysine, arginine, and have higher proportions of some essential amino acids) as opposed to the endosperm storage proteins (Metcalfe et al., 2022).

CONCLUSIONS

According to the literature, smaller wheat grains (compared across different genotypes or conditions) tend to have higher protein content and consequently higher gluten and sedimentation values. However, in the present study these quality parameters did not strictly follow the expected trend by grain size. In particular, the medium-sized grains exhibited lower protein, gluten, and sedimentation values than anticipated (the highest values were observed in the large grain fraction, while the smallest grains showed intermediate levels). This may be due to unique patterns of protein accumulation as the grain develops in different positions on the spike within the same variety. It is plausible that grains developing in the central parts of the spike (which in our case might correspond to the large-size group) achieved both large size and high protein, whereas grains from other positions (making up the medium group) did not. Further detailed studies, sampling grains by both position and size, are needed to confirm this interpretation and to generalize the findings.

Despite the unexpected dip in protein and gluten for the medium group, the obtained results did confirm, in line with the literature, that starch accumulation is higher in larger grains and that protein concentration is relatively higher in smaller grains. We also observed that the content of certain essential amino acids such as methionine+cysteine, arginine, and valine decreased as TGW increased, whereas no significant change was observed in leucine, phenylalanine, and isoleucine levels in relation to grain weight. These findings suggest that grain size can influence not only the quantity of protein and starch in wheat, but also the quality of protein in terms of its amino acid composition.

In summary, this study highlights that even within a single wheat cultivar harvested under uniform conditions, there are notable differences in quality parameters between small, medium, and large seeds. Understanding these intra-varietal variations can be important for seed grading, milling, and nutritional evaluation. For instance, separating or blending grain fractions by size might be a strategy to tailor flour blends for specific purposes (high-gluten flour vs. high-starch uses) if such quality differences are consistent. Future research should explore the mechanisms behind these differences - especially the role of spike physiology and source-sink relationships in determining grain composition. Moreover, expanding the analysis to more varieties and growing conditions would indicate how universal these relationships are. Such knowledge could contribute to wheat breeding and post-harvest processing strategies aimed at optimizing both yield and grain quality.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

STUDY ON THE DEVELOPMENT AND YIELD OF SAFFRON CROCUS (*Crocus sativus* L.) IN ORGANIC FARMING CONDITIONS IN CENTRAL SOUTHERN BULGARIA

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Abstract

The study was conducted in the period 2022-2024 in the experimental field for organic farming of the Agroecological Center at the Agricultural University, Bulgaria. The yield of fresh and dry mass of saffron crocus (Crocus sativus L.) grown under organic farming conditions was studied. To achieve the goal, a field experiment was set up, with a two-row planting scheme in three repetitions and a rate of 32 plants/m^2. Italpollina organic fertilizer was used for soil fertilization before planting. The harvesting and processing of saffron flowers was carried out manually, and the drying of the fresh mass was under controlled conditions with a temperature of 40°C for 3 hours. Meteorological conditions, including the amount of precipitation, influence the beginning of the vegetation of plants, the onset and duration of the flowering phase. The indicators of the beginning of flowering, number of flowers, yield and percentage ratio of fresh and dry mass (saffron) under organic cultivation conditions in Central Southern Bulgaria were studied.

Key words*: organic farming, Crocus sativus L, yield, saffron.*

INTRODUCTION

Saffron crocus (*Crocus sativus* L.) is a plant of the Iridaceae family, which is gaining increasing popularity as an agricultural crop in low-yielding lands as well as in foothill and mountainous areas where other crops cannot be grown (Vlahova, 2022).

The genus *Crocus* includes about 80 species in the world, 32 of which are in the flora of Turkey, 18 of which are endemic to the country. Some species, including saffron crocus, bloom in autumn, and others in spring. Both the size of the bulb and the depth of planting have an impact on the yield of stigmas (Arslan et al., 2009). According to Amirnia et al. (2013) climatic features have a significant impact on the yield components of saffron crocus, with the average values of sunshine hours compared to the average value of precipitation having a positive effect on the yield of stigmas. In spring, with an increase in air temperatures of about 20°C, the leaves of *Crocus sarivus* L. dry out. The appropriate temperature for flower formation is in the range of 23 to 27°C, with the optimum temperature being 23°C. Prolonged heat with high temperatures in summer suppresses germination and the beginning of vegetation. It occurs only in autumn when temperatures drop to between 15-17°C (Molina et al., 2005). Phenological studies are important for determining the influence of climatic features on the growth, development and flowering of crocus, which is why the BBCH scale for saffron crocus was created for the first time (Corcoles et al., 2015). Fertilization with manure increases the flower, leaf and bulb indices of saffron crocus. The combined application of manure with artificial fertilizers also increases the yield, increasing the content of microcrocin, safranal and crocin in the stigmas of *Crocus saticus* L. (Esmaeilian et al., 2022). The proven positive effect on the growth and yield of saffron after the use of manure and organic fertilizers allows the replacement of synthetic chemical fertilizers with those suitable for organic farming, which will guarantee acceptable qualitative and quantitative yields of saffron. The positive effect of summer irrigation of saffron plantations on increasing yield has also been established (Koocheki et al., 2006). When studying the effect of drying on the components of the stigmas, the duration and average temperatures for drying were established, with the most intense color being obtained at a higher drying temperature for a shorter time

(Carmona et. al., 2005). The product obtained after collecting and drying the stigmas of the saffron crocus flowers is called saffron and is the most expensive spice in the world, also known as "red gold". Saffron is rich in crocin (carotenoids) and safranal, which are the main components for the quality color, taste and aroma of saffron. The main use of saffron is in cooking due to the specific taste, aroma and color that it gives to food and drinks. It is no less valuable as a medicinal plant that has therapeutic properties on the nervous, cardiovascular system, liver (Bagur et al., 2018) and other medical purposes, thanks to the various chemical components contained in the stigmas (Bhargava, 2011).

Although saffron crocus is not particularly demanding on soil, soil type influences the formation of stamens and petals of the flower, which can be further used in other sectors, increasing income and contributing to sustainable saffron cultivation (Cardone et al., 2020b). When making extracts from saffron crocus petals, 27 phenolic compounds were found, defined as flavonoids. This allows petal extracts to be used as natural antibacterial agents (Naim et al., 2023). In his study Acero et. al. (2024) indicated that the antioxidant effect of crocus petals can be used in cosmetics, proving the positive effect against the influence of UVA radiation on the skin. This contributes to reducing the formation of spots on the skin and preserving its elasticity. The study of extracts of stigmas and petals showed that they are rich in bioactive substances and polyphenols. These compounds help fight oxidative processes in cells and protect against pathogens. These studies on the petals and the bioactive substances contained in them expand the directions for the use of saffron crocus, both in the food and in the cosmetic and pharmaceutical industries (Benkerroum et al., 2024). It has been established that the content of safranal and crocin is influenced by the processing method of raw saffron (Acar et al., 2011). A number of studies have reported the beneficial effects of saffron on the human body. Its main components have a preventive effect against tumor formations and are used in the fight against cancer and chemoprophylaxis (Abdullaev et al., 2004). The positive potential of saffron stigmas as a natural antioxidant, an alternative to synthetic antioxidants for improving the oxidative stability of soybean edible oils (Ahmed et al., 2024) has also been assessed. The antioxidant activity of saffron is also confirmed in other studies (Karimi et al., 2010), according to which the presence of gallic acid and pyrogallol was found in the stigmas.

All these valuable qualities of saffron crocus and the opportunities it offers can be a prerequisite for growing *Crocus sativus* both in areas with conditions unsuitable for growing other agricultural crops, and in organic farms to obtain ecologically clean saffron production while protecting the environment.

MATERIALS AND METHODS

The study was conducted in the period 2022-2024 in the experimental field for organic farming at the Agroecological Center at the Agrarian University, Southern Bulgaria. The Agroecological Center has been a member of the International Federation for Organic Agriculture (IFOAM) since 1993.

To achieve the goal, a field experiment was set up using the block method with saffron crocus (*Crocus sativus* L.) on the soil type Mollic Fluvisols (FAO) (Popova et al., 2012). in a two-row planting scheme (12/12/38) in three replications at a depth of 10 cm and a rate of 32 plants /m^2 with a size of the reporting plot of 3.78 m^2. Soil fertilization of the entire experimental area was carried out with Italpolina organic fertilizer (granulated bird droppings) at a dose of 70 kg/da, applied before planting in the first year. The bulbs were not removed during the three-year period. The influence of meteorological conditions (temperature and precipitation) of the year (A1 - 2022, A2 - 2023, A3 - 2024) on the yield of saffron crocus (*Crocus sativus* L.) was monitored.

The yield of fresh flowers, fresh stigmas and dried stigmas (saffron) from *Crocus sativus* L., grown under organic farming conditions for three consecutive years under non-irrigated conditions, was studied. The harvesting and processing of saffron flowers was carried out manually early in the morning, and the drying of the fresh mass was carried out under controlled conditions with a drying temperature

of 40°C for 3 hours. The statistical processing of the experimental data was carried out using SPSS V.13.0 for Microsoft Windows using the Duncan method, ANOVA.

Description of the fertilizer used: ITALPOLINA - dried, granulated poultry manure rich in: N - 4%, P_2O_5 - 4%, K_2O - 4%, MgO - 0.5%, Fe - 0.8%, B - 0.2%, C - 41%, Organic matter 70.7%, Humic acids 5%, Fulvic acids 12%, Moisture 12%, Ph7. In a short time it leads to an increase in the microbiological, physical (structure, water retention) and chemical (buffering) properties of the soil. Allowed for organic farming.

RESULTS AND DISCUSSIONS

Analysis of agrometeorological conditions during the study period

The main climatic factors affecting germination, growth, development and yield are temperatures and precipitation with their distribution during the growing season. According to Rahimi et al. (2017a) environmental factors such as precipitation, relative humidity and average annual temperature have a strong impact on the weight of the saffron bulb. The autumn of 2022 was characterized as warm and dry with temperature values above normal and a scanty amount of precipitation of only 2.1 mm/m^2 for the month of October (Table 1).

This negatively affects the germination of plants. During the growing seasons 2023 and 2024, the same trend of high temperatures above normal and low precipitation values, unevenly distributed throughout the month, was observed. In October 2022, the average monthly temperature was 16.4°C, which is nearly 4°C above the long-term norm. In 2023, the temperature values were again above normal. These high temperature values and precipitation values far below the norm for the month, negatively affect the germination, beginning and duration of flowering of saffron crocus, and hence the yield and quality of the stigmas.

On average, during the study period, the germination and flowering of saffron crocus (*Crocus sativus* L.) occurs in conditions of high temperatures and low precipitation values.

Table 1. Average monthly air temperatures and monthly amount of rainfall for the study period (2022-2024)

Months	IX	X	XI
Years	Temperature (°C)		
2022	19.4	14.1	10.0
2023	21.7	16.4	9.7
2024	21.1	13.7	6.0
Average monthly temperature- norm 1965-1995	18.3	12.6	7.4
	Rainfall (mm/m^2)		
2022	33	2.1	34
2023	19	3.2	64
2024	17	0.3	15.1
Amount of rainfall-norm 1965-1995	65	47	35

Flowering and duration of the phenological phase

The meteorological conditions of the year influence the beginning of vegetation and flowering in plants. According to Gresta et al. (2009) the beginning of flowering is influenced by the combination of temperature and soil moisture. During the study period, depending on the temperatures and the distribution of precipitation, the flowering phenological phase begins at the earliest in the third experimental year on 9.X.2024 (Table 2). The difference compared to the other two years is due to the precipitation in the second half of September, which gives rise to the vegetation of the plants, and the latest flowering begins in the dry autumn of 2022 (18.X.), supported by precipitation in the first ten days of October. The presence of moisture in the soil determines the germination and the beginning of flowering, as well as the duration of flowering in the plantation. Frequent and prolonged rainfall during flowering deteriorates the quality of flowers, stigmas and reduces yield. Depending on the distribution of precipitation and temperature values, the longest flowering of the plantation was recorded in the autumn of 2023 - 23 days, followed by 2024 and 2022 (Table 2). The plants in the young plantation form an average of one flower per season, with the largest number of flowers per unit area being obtained in the third year 2024, an average of 26.6 pcs./m^2.

Flower and stigma yield, g/1000 m²

The stigma yield of saffron crocus *Crocus sativus* L. is influenced by both weather conditions during the year and soil nutrients. According to a study by Amiri (2008), the use of cow dung + P+N fertilizers applied to the soil increases the yield of dry stigmas (dry saffron), the use of sheep dung increases the yield of bulbs, and N fertilizers increase vegetative growth. With the increasing interest in saffron production, the possibility of foliar fertilization during the winter months has been studied, which increases the number of flowers and yield by 33% (Hosseini et al., 2004). Depending on the production guidelines, a choice can be made for the most suitable fertilizer for saffron crocus. In the present study, soil fertilization was carried out with Italpolina organic poultry manure, permitted for application in organic agriculture. The use of poultry manure reduces the amount of fertilizer applied per unit area compared to other types of manure. The proven highest yield of flowers was obtained in the third year of the study (2024) of 5700 g/1000 m², with no proven difference between the yields of the previous two years (Table 3). The yield of raw stigmas (Figure 1) has a proven highest value in the dry and warm autumn of 2022 - 511 g/1000 m². In the following years, the yield varies from 380 to 414 g/1000 m², but the difference is not proven. It is noted that the conditions of the year (Table 1), in particular the distribution of precipitation before and during flowering, affect the yield of fresh stigmas. Prolonged precipitation during the flowering period, although in small quantities, makes it difficult to harvest the delicate flowers in a timely manner and leads to deterioration of quality and loss of flowers, respectively, stigma yield.

Table 2. Flowering duration and flower yield of saffron crocus (*Crocus sativus* L.) in the study period 2022-2024

Year	Beginning of flowering	End of flowering	Number of plants /m²	Total number of flower	Number of flower /m²	Number of flower per plant	Number of flower /da	Flowering duration in days
2022	18.X.	05.XI.	32	293	25.8	0.81	25838	19
2023	10.X.	01.XI.	32	288	25.4	0.80	25397	23
2024	09.X.	29.X.	32	302	26.6	0.83	26631	21
Average for the period			32	294.3	25.9	0.81	25955	21



Figure 1. Raw stigmas of saffron crocus (*Crocus sativus* L.) before drying (Personal archive: Pl. Zorovski, 2024)

This is confirmed by a study by other authors from 2019 (Cardone et al., 2019), where according to them, the place of cultivation with higher air temperatures and without excessive rainfall during flowering leads to a greater yield of stigmas with better quality, and according to Gresta et al. (2009) lower temperatures lead to greater flower production, but with lower stigma quality. In our study, after drying the raw stigmas at 40°C for 3 hours, dry stigmas (dry saffron) with a low percentage of moisture, a deep red color and thus the valuable ingredients in it are obtained.

For the study period, the proven highest yield of dry stigmas (dry saffron) was obtained in 2022 - 151 g/1000 m², followed by 2023 - 123 g/1000 m² and 2024 - 113 g/1000 m², but there is no proven statistical difference between them.

Table 3. Average yield of flowers and stigmas of saffron crocus (*Crocus sativus* L.) in the study period 2022-2024

Year	Yield g/1000 m^2			
	Fresh flower	Raw stigmas yield	Dry stigmas (saffron)	% of dry saffron compared to raw stigmas
2022	5470b	511a	151a	29.5
2023	5460b	414b	123b	29.6
2024	5700a	380b	113b	29.7
Average for th period	5540	435	129	29.6

*Means followed by the same letter are not statistically different (P<0.05) by Duncan's multiple range test

On average for the study period under the specific agroclimatic conditions and cultivation method, an average yield of raw stigmas of 435 g/1000 m^2 or 129 g/1000 m^2 of dry saffron (stigmas) was obtained. Depending on the temperature, method and time of drying, the percentage ratio of dry stigmas to the mass of raw stigmas can be predicted. At the used temperature (40°C) and drying time (3 hours), the percentage ratio of dry stigmas to the yield of raw stigmas varies within narrow limits over the years and on average for the period reaches 29.6%. Along with the deep red stigmas in the flower stalk, the base of the stigma remains, as a pale yellow to orange stigma, which can also be used as lower quality saffron. According to Naim et al., (2023), the petals of saffron crocus can also be used in the food and pharmaceutical industries.

CONCLUSIONS

The lack of precipitation and high temperatures delay the onset of flowering in saffron crocus (*Crocus sativus* L.). The longest flowering was recorded in the autumn of 2023 - 23 days, followed by 2024 and 2022. During the study period, the plants formed an average of one flower. The largest number of flowers per unit area was obtained in 2024 (average 26.6 pcs./m^2). The highest proven yield of flowers was in 2024 - 5700 g/1000 m^2. The yield of raw stigmas was proven to be the highest in the dry and warm autumn of 2022 - 511 g/1000 m^2, and in the following years the yield varied from 380 to 414 g/1000 m^2. Prolonged rainfall during the flowering period leads to loss of flowers. The proven highest yield of dry stigmas (dry saffron) was obtained in 2022 - 151 g/1000 m^2, followed by 2023 - 123 g/1000 m^2 and 2024 - 113 g/1000 m^2, between which there is no proven difference. On average for the study period under the specific conditions and organic farming, an average yield of raw stigmas of 435 g/1000 m^2 or 129 g/1000 m^2 of dry saffron (stigmas) was obtained. At a temperature of 40°C and a drying time of the stigmas of 3 hours, the percentage ratio of dry stigmas (saffron) to the raw yield of stigmas was 29.6%.

ACKNOWLEDGEMENTS

With the financial support of Project No. 17-12 "Support of Publication Activity" at the Research Center - Agricultural University - Plovdiv, Bulgaria.

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MISCELLANEOUS

Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

SURVEY ON SUSTAINABLE PROMOTION OF NUTRITIONAL STATUS OF CROPS

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Abstract

Sustainable plant nutrition is essential for maintaining soil fertility and minimizing environmental impacts. This study evaluates farmers' perceptions, challenges, and adoption of sustainable fertilization practices through a structured questionnaire. Results indicate that crop rotation is the most preferred method, while a combination of chemical and organic fertilizers is widely used. Despite moderate effectiveness, concerns about environmental impact are high, yet adoption of mitigation measures remains limited. Financial constraints, particularly the high cost of organic fertilizers, are the main barriers to sustainability. Most respondents rely on fertilizer manufacturers for information, with lower engagement in scientific research. Strong interest in training exists, though accessibility issues persist. Spearman correlation analysis highlights links between fertilization practices, awareness, and sustainability actions. Principal Component Analysis identifies key factors influencing farmers' decisions, including financial support and knowledge accessibility. The findings emphasize the need for subsidies, expert collaboration, and improved information dissemination to promote sustainable fertilization.

Key words*: environmental impact, soil fertility, principal components analysis, trends.*

INTRODUCTION

Sustainable plant nutrition is essential for maintaining soil fertility, improving crop yields, minimizing environmental impacts and creating suitable context for food security. It also plays an essential role in reducing the environmental footprint of agricultural activities (Lin et al., 2015; Muhammad et al., 2019). According to current global tendencies, it is considered that the agricultural food systems yields should align with sustainable development aims, and promote human, and animal health (El-Ramady et al., 2020; Six, 2011; White & Brown, 2010).

It involves the dissemination of knowledge, implementation of best practices, and support for policies that encourage the adoption of eco-friendly fertilization methods (Meisterling et al., 2009).

As agricultural practices evolve, the balance between productivity and sustainability becomes increasingly important, requiring a shift toward integrated fertilization strategies that combine chemical and organic inputs (Chandel et al., 2024). While crop rotation and controlled fertilization have been widely adopted, challenges such as high costs, limited awareness, and accessibility to sustainable alternatives continue to be critical points in the widespread implementation of environmentally friendly practices (Atz et al., 2019; Godfray et al., 2010; Gomeiro et al., 2011).

Fertilization practices are limitative in soil health and nutrient availability, but excessive reliance on chemical products has raised concerns about soil degradation, water contamination, and greenhouse gas emissions (Lal, 2015; Savci, 2012). Consequently, regenerative agriculture and organic fertilization are gaining attention as viable solutions, though their adoption remains limited due to financial constraints and a lack of technical knowledge (Khangura et al., 2023; Schreefel et al., 2020). Additionally, the primary sources of information on plant nutrition, predominantly fertilizer manufacturers, may influence farmers' choices, limiting exposure to scientific research and sustainable alternatives. Understanding farmers' perceptions and challenges in adopting sustainable fertilization methods is critical for designing effective policies and support programs (Adnan et al., 2019; Sun et al., 2022).

This study aims to analyze the current trends in plant nutrition practices, assess the awareness of

sustainability issues, and identify the key factors influencing the adoption of regenerative fertilization strategies. By evaluating the relationships between fertilization practices, environmental concerns, and access to knowledge, this research seeks to provide insights that can guide future initiatives in promoting sustainable agricultural development.

MATERIALS AND METHODS

To emphasize the role of sustainable promotion of plant nutritional status in enhancing production of farmers focused on vegetal production a survey was conducted, during April - October 2024. Data were collected through a structured questionnaire, which included multiple-choice questions designed to capture respondents' preferences, awareness, and constraints in adopting sustainable fertilization practices. The questionnaire covered key topics such as plant nutrition methods, fertilizer types, effectiveness evaluation, environmental concerns, information sources, training interest, and perceived barriers.

The survey targeted agricultural practitioners, including farmers, agricultural consultants, students in agronomy, and researchers, to ensure a diverse representation of expertise and perspectives. The sample was selected using a stratified random sampling approach, ensuring coverage of different farm sizes and cultivation practices, including conventional and organic farming. The questionnaire was distributed through direct interviews, with a total of 400 valid responses collected. The sample size was calculated using the Cochran formula (https://www.statisticshowto.com/probability-and-statistics/find-sample-size/).

The collected data were analyzed using descriptive statistics to determine response frequencies and trends. To identify relationships between variables, Spearman's rank correlation coefficient was applied, assessing the associations between fertilization practices, environmental concerns, and access to knowledge. Principal Component Analysis (PCA) was employed to reduce data dimensionality and identify key factors influencing farmers' decisions regarding plant nutrition. PCA opportunity was tested by applying the Bartlett and Keiser - Meyer - Olkin (KMO) tests (Merce & Merce, 2009). Statistical analyses were performed using Statistica v.8 for windows.

RESULTS AND DISCUSSIONS

Equipping farmers globally with crop rotation management tools could be a generous solution in achieving the sustainable development goals (Schöning et al., 2023). Crop rotation practices are the most preferred method, with 65% of respondents using this approach (Figure 1). This suggests that many farmers recognize the long-term benefits of crop rotation in enhancing soil fertility and plant nutrition. We consider that more awareness and education programs could encourage farmers to adopt organic fertilizers and biostimulants. We also consider that policymakers and agricultural support programs could incentivize the adoption of organic methods to improve soil health and reduce dependency on chemical fertilizers.

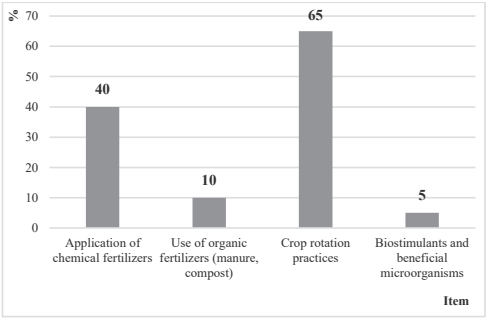


Figure 1. The respondents' opinions concerning the methods used to improve plant nutrition

There are studies showing that applying a combination of chemical and organic fertilizers enhances soil quality, supports plant growth, and increases yield compared to using chemical fertilizers alone (Wan et al., 2021). A combination of both chemical and organic fertilizers is the most commonly used approach, adopted by 50% of respondents (Figure 2). This indicates a balanced approach to plant nutrition, leveraging the benefits of both synthetic and natural inputs. The preference could suggest that many respondents recognize the advantages of integrating organic matter for soil health while still relying on synthetic fertilizers for immediate plant nutrition.

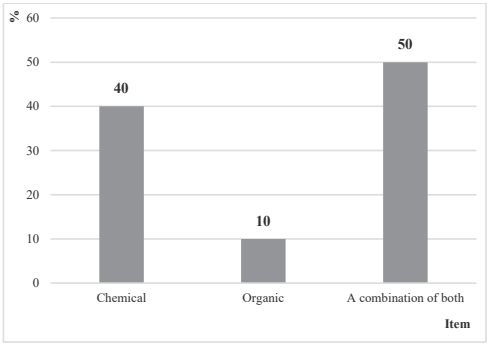


Figure 2. The respondents' opinions concerning the fertilizers used most often

Majority of interviewed subjects, 90% respectively, consider their plant nutrition practices to be moderately effective, indicating that while they observe positive results, there is still potential for improvement, while only 10% rate their practices as very effective (Figure 3). It results that few practitioners feel completely confident in their approach, and none of the respondents consider their methods ineffective, implying that all observe at least some benefits from their strategies, though further optimization and innovation could enhance overall effectiveness.

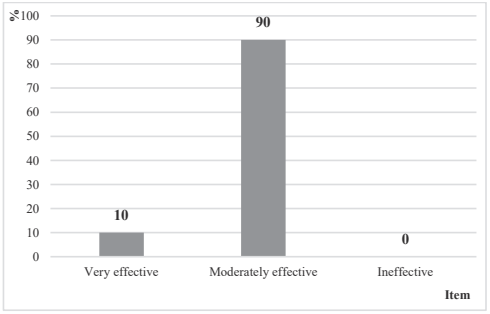


Figure 3. The respondents' opinions concerning the evaluation of the effectiveness of plant nutrition practices

90% of respondents express concern about the environmental impact of fertilization, indicating a high level of awareness regarding sustainability issues in agricultural practices (Figure 4). Only 10% do not share this concern, suggesting that although most acknowledge the potential risks associated with fertilization, there is still a small proportion that may require further information or motivation to adopt more environmentally friendly approaches.

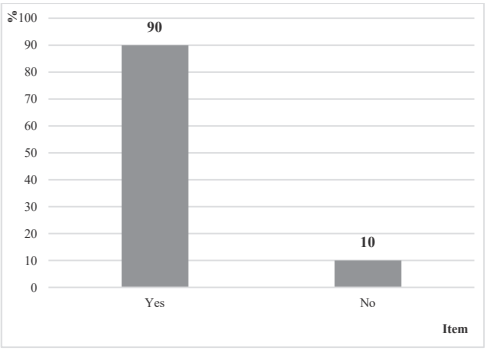


Figure 4. The respondents' opinions regarding the concerns about the environmental impact of fertilization

A half of the respondents have not yet taken any measures to minimize the negative impact of fertilization on soil and water, suggesting a significant gap in the adoption of sustainable practices (Figure 5). 35% implement controlled application of fertilizers, reflecting a more precise approach to nutrient management, only 10% alternate fertilizer types to reduce chemical dependency, and a minimal 5% use soil sensors to monitor nutrients. These findings suggest the need for greater awareness and accessibility to advanced monitoring technologies.

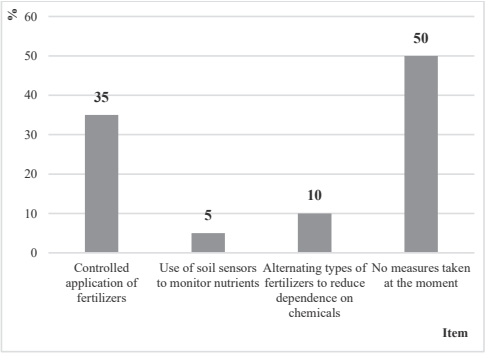


Figure 5. The respondents' opinions concerning the measures to minimize the negative impact of fertilization on soil and water

Majority of interviewed subjects, 80%, have only partial awareness of the benefits of regenerative agriculture for plant nutrition, indicating that while some knowledge exists, there is still a need for more comprehensive education and outreach (Figure 6). 15% are fully aware, suggesting a smaller group actively engaged with these principles, and only 5% have no awareness, showing that most respondents

recognize at least some aspects of regenerative agriculture but may require further guidance to fully integrate its practices.

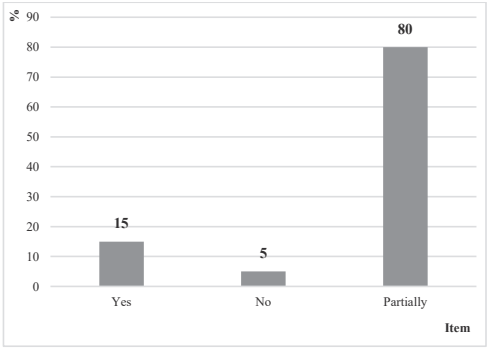


Figure 6. The respondents' opinions concerning the awareness of the benefits of regenerative agriculture for plant nutrition

60% of questioned subjects rely on fertilizer manufacturers as their primary source of information about plant nutrition and fertilization, suggesting a strong influence of industry-driven knowledge, while 30% seek advice from agricultural consultants (Figure 7). 20% depend on their own experience, reflecting a hands-on learning approach, 15% obtain information from researchers and universities, showing a relatively lower engagement with scientific research, and only 10% use the internet and social media, suggesting limited reliance on digital sources for agricultural knowledge.

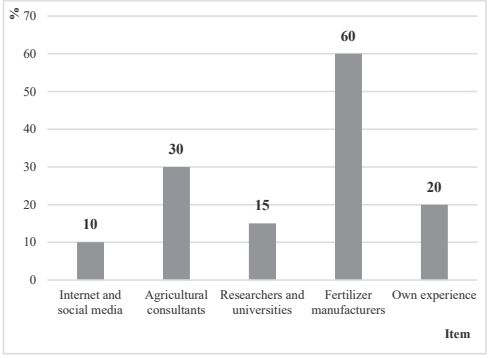


Figure 7. The respondents' opinions concerning the sources of information about plant nutrition and fertilization

A proportion of 75% respondents, express interest in participating in training or workshops on sustainable crop fertilization, indicating a strong willingness to enhance their knowledge and adopt improved practices (Figure 8). In the meantime, 20% are open to the idea but consider cost and availability as determining factors, suggesting that accessibility plays an important role in participation, and only 5% are not interested.

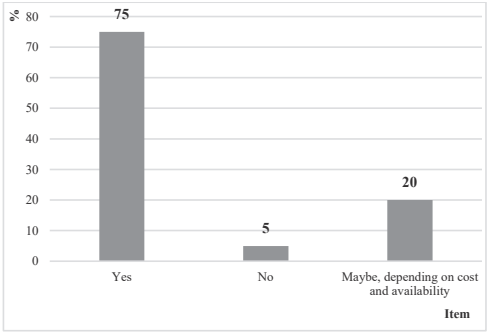


Figure 8. The respondents' opinions concerning the interest in participating in training or workshops on sustainable crop fertilization

Majority of respondents, 80%, respectively, identify the high costs of organic fertilizers as the main challenge in adopting sustainable fertilization practices (Figure 9). This finding could suggest that financial constraints are a significant barrier. 20% of interviewed persons mention the lack of clear information, suggesting that improved access to knowledge and guidance could facilitate adoption, and only 10% report encountering no challenges. The responses show that most respondents face obstacles that hinder the transition to more sustainable fertilization methods.

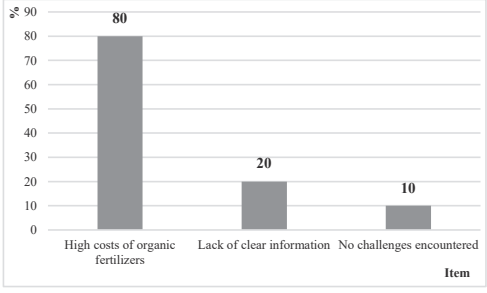


Figure 9. The respondents' opinions concerning the challenges faced in adopting sustainable fertilization practices

90% of the questioned subjects consider financial support or subsidies as the most

necessary form of assistance for adopting more sustainable practices, while 25% emphasize the importance of collaboration with experts in the field, and 20% express the need for both access to information and practical guides as well as access to innovative products (Figure 10). These responses suggest the major role of technical guidance.

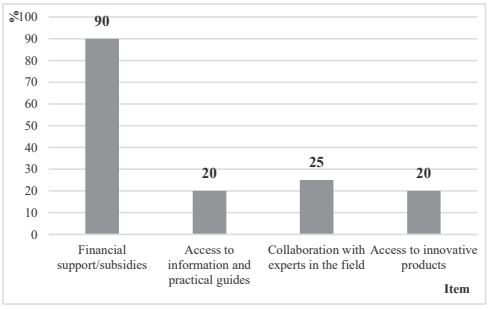


Figure 10. The respondents' opinions concerning the support needed to adopt more sustainable practices

The simple Spearman correlations between various variables related to the sustainable promotion of plant nutritional status, showing that the methods used to improve plant nutrition (Var1) exhibit a moderate positive correlation with the evaluation of plant nutrition effectiveness (Var3) and concerns about the environmental impact of fertilization (Var4), suggesting that better nutrition practices are linked to higher awareness and perceived effectiveness (Table 2). The fertilizers used most often (Var2) have strong negative correlations with measures to minimize the negative impact of fertilization (Var5), awareness of regenerative agriculture benefits (Var6), and sources of information (Var7), indicating that reliance on certain fertilizers may be associated with lower engagement in sustainable strategies. The strong positive correlations between measures to minimize fertilization impact (Var5), awareness of regenerative agriculture (Var6), and participation in training (Var8) suggest that those aware of sustainability principles are more likely to act. The challenges in adopting sustainable fertilization practices (Var9) and the support needed (Var10) show a perfect correlation, reinforcing that financial and informational constraints significantly impact adoption rates.

Table 2. The simple Spearman correlations between the variables concerning the sustainable promotion of plant nutritional status

Issue	Var1	Var2	Var3	Var4	Var5	Var6	Var7	Var8	Var9	Var10
Var1	1.00	-0.16	0.62	0.62	0.57	0.62	0.41	0.65	-0.05	-0.05
Var2	-0.16	1.00	-0.45	-0.45	-0.87	-0.70	-0.91	-0.59	-0.82	-0.82
Var3	0.62	-0.45	1.00	1.00	0.62	0.72	0.66	0.76	0.30	0.30
Var4	0.62	-0.45	1.00	1.00	0.62	0.72	0.66	0.76	0.30	0.30
Var5	0.57	-0.87	0.62	0.62	1.00	0.88	0.92	0.82	0.66	0.66
Var6	0.62	-0.70	0.72	0.72	0.88	1.00	0.88	0.95	0.47	0.47
Var7	0.41	-0.91	0.66	0.66	0.92	0.88	1.00	0.77	0.69	0.69
Var8	0.65	-0.59	0.76	0.76	0.82	0.95	0.77	1.00	0.39	0.39
Var9	-0.05	-0.82	0.30	0.30	0.66	0.47	0.69	0.39	1.00	1.00
Var10	-0.05	-0.82	0.30	0.30	0.66	0.47	0.69	0.39	1.00	1.00

Var1 - Methods used to improve plant nutrition; Var2 - Fertilizers used most often; Var3 - Evaluation of the effectiveness of plant nutrition practices; Var4 - Concerns about the environmental impact of fertilization; Var5 - Measures to minimize the negative impact of fertilization on soil and water; Var6 - Awareness of the benefits of regenerative agriculture for plant nutrition; Var7 - Sources of information about plant nutrition and fertilization; Var8 - Interest in participating in training or workshops on sustainable crop fertilization; Var9 - Challenges faced in adopting sustainable fertilization practices; Var10 - Support needed to adopt more sustainable practices.

The implementation of PCA reveal that the first principal component, accounting for 66.634% of the variance, is associated with practices used for plant nutrition, where fertilizers used most often show a strong positive factor loading of 0.8475, while methods used to improve plant nutrition and the evaluation of their effectiveness have negative loadings, suggesting that the choice of fertilizers influences perceptions more than the actual methods or perceived effectiveness (Table 3).

Table 3. The principal componets analysis on variables of the sustainable promotion of plant nutritional status

Eigenvalue	Variance, %	Factor	Item	Factor loading
6.6634	66.634	Practices used for plant nutrition Mean = 2.267 SD = 0.944	Methods used to improve plant nutrition	-0.5557
			Fertilizers used most often	0.8475
			Evaluation of the effectiveness of plant nutrition practices	-0.7935
2.0988	20.988	Sustainability and environmental impact Mean = 2.233 SD = 0.916	Concerns about the environmental impact of fertilization	-0.7935
			Measures to minimize the negative impact of fertilization on soil and water	-0.9479
			Awareness of the benefits of regenerative agriculture for plant nutrition	-0.9259
1.2378	12.378	Perception and access to information Mean = 1.900 SD = 0.481	Sources of information about plant nutrition and fertilization	-0.9469
			Interest in participating in training or workshops on sustainable crop fertilization	-0.8821
			Challenges faced in adopting sustainable fertilization practices	-0.6879
			Support needed to adopt more sustainable practices	-0.6879

The second component, explaining 20.988% of the variance, relates to sustainability and environmental impact, with strong negative loadings for concerns about fertilization's impact, measures to mitigate these effects, and awareness of regenerative agriculture, indicating that higher awareness is linked to increased concern and action. The third component, covering 12.378% of the variance, pertains to perception and access to information, where sources of information, interest in training, and challenges in adopting sustainable practices all have strong negative loadings, suggesting that greater access to knowledge and participation in educational programs are associated with a heightened recognition of challenges and the need for support (Table 3).

CONCLUSIONS

The findings highlight crop rotation as the preferred method for plant nutrition, while the combined use of chemical and organic fertilizers reflects a balanced approach. Despite moderate perceived effectiveness, there is potential for optimization, with sustainability concerns widely acknowledged but not always addressed in practice. Limited awareness of regenerative agriculture underscores the need for educational initiatives. Reliance on fertilizer manufacturers as a primary information source suggests industry influence, while low engagement with scientific research presents opportunities for diversified knowledge access. Strong interest in training contrasts with financial and accessibility barriers, with the high cost of organic fertilizers being the main obstacle. Financial support, expert collaboration, and improved information dissemination are essential for promoting sustainable fertilization practices.

The correlation analysis reveals that improved plant nutrition methods are linked to higher awareness and perceived effectiveness, while reliance on certain fertilizers is associated with lower engagement in sustainability strategies. The principal component analysis confirms that plant nutrition practices, sustainability concerns, and access to information are the key factors influencing perceptions of carbon sequestration, with financial and technical support playing a crucial role in driving sustainable agricultural transitions.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

A COMPARATIVE STUDY ON THE EVOLUTION OF PLANTED BLACK PINE SAPLINGS ON THE STERILE DUMPS FROM RECEA ȘUNCUIUȘ QUARRY, BIHOR COUNTY

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Abstract

This study analyses the performance of black pine (Pinus nigra Arn.) seedlings planted in 2008-2009, of local origin, and those planted in 2023, with Austrian origins, on the waste dumps of the Recea Șuncuiuș Quarry in Bihor County. The survival rate of the seedlings was assessed, and their diameters and heights were measured across different slope categories. Preliminary results show that the seedlings planted in 2023 exhibit strong growth in both height and diameter, particularly on medium slopes. The overall survival rate of the seedlings planted in 2008-2009 was around 84%. The study also examines the impact of pests on the black pine stand on the Recea Quarry waste dump. Lastly, the health of the trees planted in 2008-2009 was assessed.

Key words*: afforestation, health status, pine, Pinus, survival rate.*

INTRODUCTION

Ecological restoration is a process aimed at rehabilitating ecosystems impacted by human activities or other disturbances. This can involve restoring natural habitats, reintroducing native species, managing natural resources sustainably, and implementing various strategies to restore balance and enhance biodiversity (Miyawaki & Golley, 1993; Stanturf et al., 2014; Chen et al., 2024).

The primary goal of ecological restoration is to recreate a healthy, functioning environment that sustains life and benefits both nature and human communities. This process can be complex, often requiring collaboration among stakeholders, including academia, research institutes, governments, non-governmental organizations, local communities, and conservation experts (Reid et al., 2021; Schweizer et al., 2021).

Afforestation represents one of the main components of ecological restoration worldwide (Cui et al., 2021; Korneeva, 2021). Pine species, especially black pine (*Pinus nigra* Arn.) and Scots pine (*Pinus sylvestris* L.) are among the most planted tree species worldwide (Enescu, 2015; Vacek et al., 2021; Yildiz et al., 2022).

Over time, the primary purpose of conifer plantations has evolved: whereas they were originally established primarily for wood production, today they are crucial for protecting soil and water resources and for restoring degraded forests (Tavankar et al., 2018; Constandache et al., 2021). Pines are among the most widely planted species globally (Malkamäki et al., 2018; McEwan et al., 2020; Uribe et al., 2020), accounting for 40% of all plantations worldwide (Brown & Ball, 2007).

Pines are the dominant tree species across large areas of the Northern Hemisphere, but over the past few centuries, human activities have shaped the distribution, composition, and structure of pine forests (Richardson et al., 2007).

As a result, black pine now has a fragmented distribution range that extends from North-Western Africa, through southern Europe, to Asia Minor (Kaya & Temerit, 1994; Enescu et al., 2016).

Currently, black pine occupies over 3.5 million hectares (Isajev et al., 2004), making it one of the most widely distributed conifer species in the Balkans and Asia Minor. Its largest global range is in Turkey, where it covers more than 2.5 million hectares (Sevgi & Akkemik, 2007).

In recent decades, black pine has been extensively used in Romania for the afforestation of degraded lands (Crăciunescu et al., 2014; Silvestru-Grigore, 2018; Tudoran et al., 2023).
The main objective of this paper was to assess the growth of black pine seedlings planted in 2023 in Recea Șuncuiuș Quarry (Bihor County). Secondly, the vegetation status of the pines planted in 2008-2009 in the same area was assessed.

MATERIALS AND METHODS

The experiments were carried out within the 2.89 km² Recea perimeter, located in the Șuncuiuș refractory clay deposit in the northern part of the Pădurea Craiului Mountains, Bihor County (46°55'11"N, 22°30'34"E).
According to Annex 2 of Ministerial Order no. 2533/2022 - Guide to Good Practices for Compositions, Schemes, and Technologies for Forest Regeneration and Afforestation of Degraded Lands (MMAP, 2022), the type of landfill site in the analyzed case is classified as YD1B. The symbol represents: Y for landfill; D for hill region; 1 for raw waste dumps or terrigenous material resulting from mining, geological exploration, excavations, or various digging activities; and B for anthropogenic material composed of small (coarse or fine) materials such as sand, gravel, loess, and clay.
This annex also provides the afforestation compositions and planting schemes for these types of degraded lands.
In March-April 2023, four experimental plots, each measuring 50 m² were established within the perimeter (Figure 1).

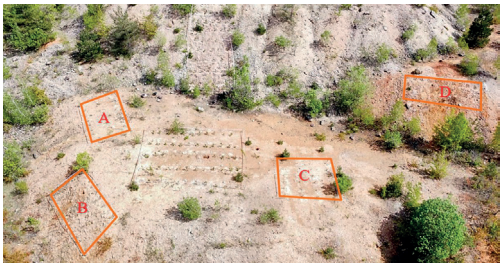


Figure 1. Location of the 4 experimental plots

One-year-old black pine seedlings were planted in these plots, following a 2x1m planting pattern (2 meters between the rows and 1 meter between the seedling in the same row), with 25 seedlings per plot.
Additionally, five seedlings of common hazel (*Corylus avellana* L.) were planted in each plot. Hazel seedlings were selected due to the rapid decomposition of their leaves, which release micronutrients such as Sulphur (S), Calcium (Ca) and Potassium (K), essential for plant growth (Laekemariam & Elka, 2022; Bolat et al., 2025). This process also increases soil organic matter, enhancing soil structure and water retention capacity (Gupta & Malik, 1999), diminishing at the same time the runoff and other associated processes of land degradation.
The experimental plots were established in lands where natural regeneration has not taken place, and previous attempts at artificial regeneration have been unsuccessful, except for some Scots pine specimens planted 10-12 years ago.
These lands consist of a terrace created by levelling the waste rock from clay extraction, along with the adjacent slopes. In 2016, black locust (*Robinia pseudoacacia* L.) saplings were planted on the terrace, but they failed to adapt due to excess of moisture.
The slopes chosen for the experiment have a steep gradient of 30-40°, and previous plantings in these areas have produced very poor results.
The four experimental fields were labelled as follows (Figure 2):

- Variant A, code Pi.n. S1P1N: low slope P1≤10°, N - unfertilized substrate;
- Variant B, code Pi.n. S2P2N: large slope P2>10°, N - unfertilized substrate;
- Variant C, code Pi.n. S3P1F: low slope P1≤10°, F - fertilized substrate;
- Variant D, code Pi.n. S4P2F: large slope P2>10°, F - fertilized substrate.

Typically, seedlings are planted in regular pits (30/30/30 cm), as recommended by specialized literature on establishing pure pine plantations (Traci & Untaru, 1986).
However, in this case, enlarged regular pits (40/40/40 cm) were used because, although the root system volume of the seedlings was small, an additional nutrient substrate is necessary for degraded lands.
Immediately after planting, each seedling was provided with 5 liters of water in each pit.

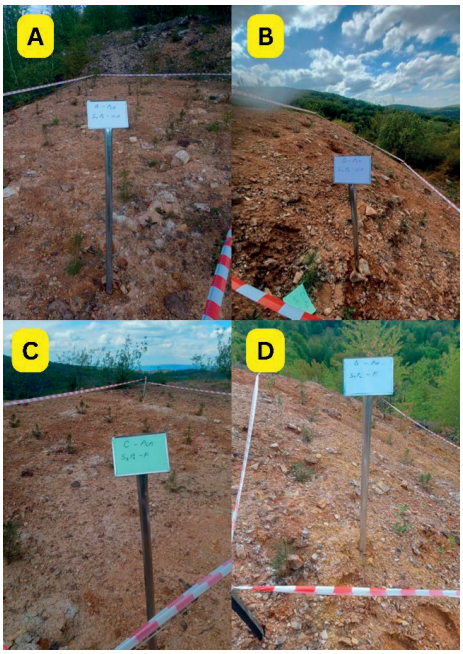


Figure 2. Details regarding the 4 experimental plots

For planting, black pine seedlings, sourced from Austria, and grown in containers measuring 70 mm x 100 mm were used (Figure 3).



Figure 3. Seedlings grown in containers

The holes were dug manually with a spade and pickaxe to remove any boulders. Borrowed soil was then placed at the bottom of each hole, and the seedling was positioned in the center of the holes. Around the seedling's roots, alternating layers of topsoil, acidic peat, and sterile peat with high clay content were added. This mixture helps reduce water evaporation during dry periods and aids the seedling in acclimating to the new soil conditions more quickly.

In the experimental plots located on very steep slopes, the soil was stabilized with dry stone masonry to prevent waste rock from blocking the seedlings over the winter. Fertilization was applied immediately after planting to half of the experimental plots, using a fertilizer designed for softwood seedlings with the following composition: 14% N, 7% P_2O_5, 17% K_2O, 2% MgO, 9% S, 0.02% B, 0.01% Zn. The fertilizer was spread around the planted seedlings, with 20 grams applied to each seedling (Figure 4).



Figure 4. Applying fertilizers

In the third decade of April 2023, at the start of the first year of vegetation, observations were made to assess the onset of seedling growth (the

degree of survival after planting). The following characteristics were measured:

- the degree of survival after planting in the field, expressed as a percentage;
- the degree of seedling survival, recorded at the end of the first vegetation period, expressed as a percentage;
- seedling height (cm), measured from ground level to the top of the main stem;
- seedling diameter at the root collar (mm) at the end of the first vegetation year in the field;
- the number of whorls;
- the number of branches per whorl.

The diameter at the root collar was measured using a digital caliper (Figure 5), and the height was measured with a height measuring tape (Figure 6).



Figure 5. Measuring the root collar with a digital caliper

For most of the analyzed characteristics (degree of survival after planting, degree of survival after the first year, plant height, diameter at the root collar, number of whorls, and number of branches per whorl), the arithmetic mean was calculated using the standard formula: $x = \sum x/n$.



Figure 6. Measuring the height with a measuring tape

For the number of branches per first whorl, as each sample contained groups of plants with varying numbers of branches (ranging from 0 to 6 branches per whorl per plant), the weighted arithmetic mean was used, calculated with the following formula:

$$X_{med.pond.} = \frac{\bar{x}_1 n_1 + \bar{x}_2 n_2 + \ldots + \bar{x}_k n_k}{N},$$

in which: $N = n_1 + n_2 + \ldots + n_k$;

$\bar{x}_1 \cdots \bar{x}_k$ = group averages.

To determine the degree of survival after planting in the field, the following formula was used:

$$Gp(\%) = \frac{Pveg}{Pplant}\ 100,$$

where: P_{veg} represents seedlings started in vegetation on April 20, 2023;

P_{plant} accounts for planted seedlings.

To determine the degree of survival after planting in the field, the following formula was used:

$$Gm(\%) = \frac{Pviab}{Pveg}\ 100,$$

where: Pviab represents viable seedlings at the time of determination;

Pveg represents the number of seedlings that started growing on April 20, 2023.

The results from the biometric measurements of the mentioned characteristics were statistically analyzed using analysis of variance, appropriate for monofactorial experiments conducted in randomized blocks. The significance of the differences between the two tested varieties was determined using the DL test or the multiple comparisons test ($DS_{5\%}$). For certain characteristics, the "t" test (Student) was also applied (Ardelean et al., 2005; Ardelean, 2011). The coefficients of variability (s%) were calculated using data from the series of measurements taken on the 25 individuals in each variant. The applied formula was (Ardelean, 2011):

$$s\% = \frac{s}{\bar{x}}\ 100, \%$$

where: s represents the standard deviation;

x is the average of the series.

Simple correlation coefficients were calculated using Microsoft Excel, with their significance assessed at the 5% and 1% levels.

A secondary objective of the research was to evaluate the health status of the saplings planted in 2008-2009 in the same area, that recorded in 2023 an 84% survival rate (Bodea et al., 2023). The study was conducted, in January 2025, according to the recommendations of Technical Norms No. 6 – Technical Standards and Guidelines for Forest Protection (MAPAM, 2003). Defoliation and discoloration were expressed in percentages (from 5 to 5). Mechanical (physical) damages were grouped as follows: Animals, Insects, Fungi, Abiotic, Anthropogenic, Other damages. Their intensity was noted with the following classes: 0 - no damage, 1 - weak damage, 2 - medium damage, 3 - strong damage.

RESULTS AND DISCUSSIONS

Measurements taken on all seedlings prior to planting confirmed their classification into quality classes I and II based on stem height and average diameter at the root collar. The detailed results of these measurements, conducted in March and November 2023, are presented in Table 1.

The average diameters measured for all 100 seedlings at the beginning and at the end of the 2023 growing season are given in Figure 7.

Table 1. Mean values of collar diameter and heigh recorded in March and November 2023

Plot	Collar diameter [mm]		Height [cm]	
	March	November	March	November
A	9.53	9.92	9.28	30.79
B	8.44	8.70	8.40	25.79
C	9.21	10.03	8.89	30.78
D	8.62	8.89	9.52	25.04
Mean	8.95	9.39	9.02	28.10

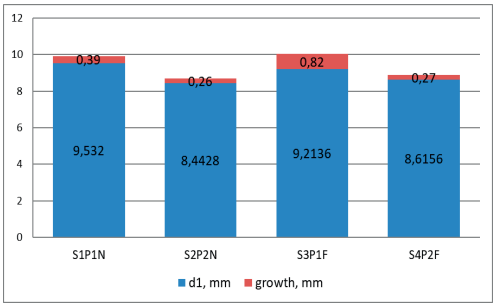


Figure 7. Average diameters measured at planting (d_1) and growth in 2023

Figure 8 presents the average heights of all 100 seedlings measured at the start and end of the 2023 growing season.

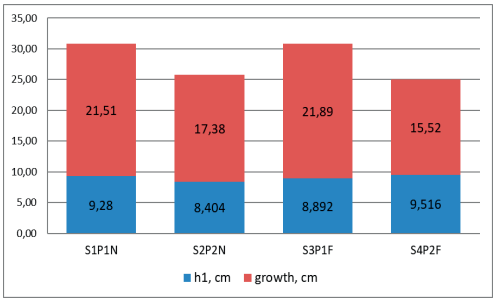


Figure 8. Average heights measured at planting (h_1) and growth in 2023

The primary data, recorded for 25 individuals from variant A (no slope, unfertilized), indicate that the seedlings' heights at the end of the first growing season ranged from 22 to 42 cm, with a coefficient of variation of 14.8%, reflecting medium variability.

The calculation and interpretation of results from the bifactorial experiments involve the following factors:

- Factor A: Fertilization method, with two levels: N (unfertilized) and F (fertilized).

- Factor B: Slope, with two levels: P1 (0-10°) and P2 (>10°).

The experiment was conducted using a randomized block design with 25 replications (n = b = 25). The total number of experimental variants (v = 4) was determined by multiplying the levels of the two factors (2 x 2 = 4).
Each plot consisted of 25 seedlings, and the average height growth for each replication area was calculated by averaging the measurements taken from all 25 seedlings within that plot.
Analysis of variance for the bifactorial experiment (2 x 2) with slope and fertilization mode is given in Table 2.

Table 2. Analysis of variance for the bifactorial experiment (2 x 2) with slope and fertilization mode

Cause of variability	SP	DF	s^2	Sample F			
Total	2223.24	99					
Blocks	544.0196	24				5%	1%
Fertilization	13.7641	1	13.76	1.05	<	3.98	7.01
Slope	689.0625	1	689.06	52.50	>	3.98	7.01
Fertilization x slope	31.47	1	31.47	2.40	<	3.98	7.01
Error	944.92	72	13.124				

According to the data from Table 2 it can be seen that the calculated F values for slope exceed the theoretical F values at the $P_{5\%}$ level. This indicates that the slope factor significantly influences seedling height growth in the first year after planting.
The significance of the differences between the variants is assessed in the following. Since multiple types of comparisons can be made, the analysis of variance method enables the calculation of s_d and LSD (Least Significant Difference) values for each comparison. In Table 3, the variants are compared to a control variant.

Table 3. Summary of the results

No. Var	Variant name	hm, cm	h rel, %	± d, cm	Significance of difference
11	NP1 (Mt)	21.5	100.0	-	-
12	NP2	17.4	80.8	-4.1	ooo
21	FP1	21.9	101.8	0.4	-
22	FP2	15.5	72.1	-6.0	ooo

LSD 5%= 2.04
LSD 1%= 2.72
LSD 5%= 3.51

A highly significant difference in seedling height growth was observed on a steep slope compared to the control variant (unfertilized NP1 on a gentle slope).
As for post-planting survival, the losses were minimal, with only 1% (1 seedling out of 100) drying out.
The health status of the 124 saplings planted in 2008-2009, in the same area is given in Table 4.

Table 4. Assessment of the health status of trees planted in 2008-2009

Slope categories	No. of sampling plot	Mechanical (physical) injuries, injury degree 0-3						Defoliation [%]	Discoloration [%]
		Animals	Insects	Fungi	Abiotic	Anthropogenic	Others		
slope < 5	S1	-	0.00	1.67	0.00	-	-	26.11	31.11
	S2	-	0.10	2.05	0.10	-	-	32.38	36.43
	Mean	-	0.05	1.86	0.05	-	-	29.25	33.77
slope 5…15	S1	-	0.06	1.56	0.17	-	-	25.00	39.50
	S2	-	0.14	1.68	0.14	-	-	25.91	31.14
	Mean	-	0.10	1.62	0.15	-	-	25.45	35.32
slope > 15	S1	-	0.04	1.42	0.13	-	-	1.42	20.83
	S2	-	0.10	1.76	0.14	-	-	27.38	32.62
	Mean	-	0.07	1.59	0.13	-	-	14.40	26.73

The degree of damage caused by insects is very low, between 0.05-0.10 (on a scale from 0 to 3), the maximum being recorded on surfaces with medium slopes. Similar results were recorded also in the case of the damage due to abiotic factors, the values being between 0.05 and 0.15. The presence of fungi led to the appearance of weak to medium damages, respectively 1.59 on

large slopes and 1.86 on small slopes (on a scale from 0 to 3).
Regarding the degree of defoliation and discoloration, the observed values are shown in Figure 9.

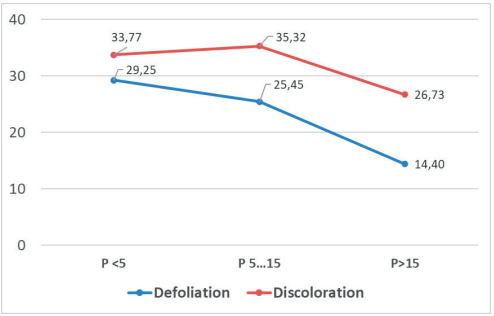


Figure 9. Degrees of defoliation and discoloration of trees (in %) on the 3 slope categories (P)

CONCLUSIONS

Black pine seedlings of Austrian origin planted in 2023 have a high survival rate (99%) compared to those from 2008-2009, where the degree of survival after 13-14 years after planting was 72-96%, depending on the slope, as it was previously reported (Bodea et al., 2023).
Although the one-year-old seedlings planted in 2023 were grown in pots (containers) and the planting holes were relatively large (40 x 40 x 40 cm), the fertilizers applied to half of the seedlings did not impact their growth in diameter or height. However, the slope was a significant factor that influenced growth.
In the case of the trees planted in 2008-2009, and analysed in January 2025, the average degree of tree damage caused by fungi was 1.69 on a scale from 0 to 3. They exhibit symptoms of successive or complex diseases, resulting from the simultaneous presence of multiple pathogens, including *Dothistroma pini* Hulbary, *Lophodermium pinastri* (Schrad.) Chevall., *Sphaeropsis sapinea* (Fr.:Fr) Dyko & Sutton in Sutton, and *Cyclaneusma minus* (Butin) DiCosmo, Peredo and Minter.
These combined infections contribute to the gradual deterioration of the trees and may accelerate the decline of pine trees in the Recea Mine tailings dump.
On the small slope, the degree of defoliation is the highest, namely 29.25%. Needle discoloration is more pronounced and affects pine specimens, the degree of discoloration being over 33% on small and medium slopes.
The main factors limiting their development are drought and the deficiency of nutrients in the soil. In addition, the attack of foliar fungi reduces the ability of trees to carry out photosynthesis, which leads to a lower intake of nutrients, slower growth and increased vulnerability to stress factors.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

RESEARCH ON PRODUCTIVITY INDICES IN SOME DEMONSTRATION BATCHES OF AUTUMN WHEAT, AT SCDA BRAILA, IN THE LAST TWO AGRICULTURAL YEARS

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Abstract

At SCDA Braila, demonstration batches of autumn wheat are organized every year, with Romanian and foreign varieties, to formulate recommendations on cultivation technologies and the choice of the best performing varieties for farmers. This paper presents the productivity elements of 25 varieties of autumn wheat cultivated in the pedoclimatic conditions of the Braila Plain, in the last two agricultural years. A classification was made regarding the productivity of the 25 wheat varieties tested in the last agricultural years and recommendations were formulated for the farmers in this area. It is essential to explore solutions to reduce operational costs, diversify crops to have a market for the products obtained and invest in advanced technologies that can increase the efficiency and sustainability of agriculture.

Key words*: wheat, productivity, technology, climatic conditions, wheat varieties.*

INTRODUCTION

Wheat kernels are used primarily to produce flour, for the manufacture of bread, pasta, and pastries, but also as a raw material to produce starch, ethyl alcohol and bioethanol.
Wheat can also be used as animal feed, having a higher nutritional quality than corn.
After the industrialization of wheat grains by milling, the resulting bran can be used as concentrated feed, being extraordinarily rich in proteins and mineral salts.
The residual straw after wheat harvest represents a coarse feed, being rich in fiber, but can also be used in the manufacture of cellulose and as bedding for animals in livestock farms.
From an agronomic point of view, winter wheat is a good precursor for all species, being harvested early and leaving the land clean of weeds and the soil with an increased amount of organic matter.
In temperate zones, wheat has become the most important staple crop, with increasing demand in countries undergoing urbanization and industrialization.
Thus, wheat is considered a major source of starch and energy, but it also provides particularly important amounts for health, such as protein, vitamins, fiber, and other nutrients that can reduce cardiovascular disease, type 2 diabetes, and various forms of colorectal cancer (Shewry, 2015).
From a genetic point of view, winter wheat has a high heritability regarding the composition of beneficial elements in the caryopsis, so that plant breeders can choose to increase certain components in breeding programs, in addition to increased crop yield.
The wheat caryopses comprise three major groups of components, represented by starch, proteins, and cell wall polysaccharides (dietary fiber) and several other components beneficial to human health.
Detailed studies conducted on dietary fiber and other bioactive components in wheat grains, in 150 wheat varieties cultivated within the EU FP6 HEALTHGRAIN program, have demonstrated that the structure and chemical composition of wheat is clearly superior to other related species, such as barley, rye and oats, including transgenic species (Shewry, 2015).
Wheat is among the oldest and most extensively grown of all grain crops (Wrigley, 2009).

According to archaeological studies, wheat is believed to have been cultivated for more than 9,000 years, with its origins traced to southeastern Türkiye.
Its name was Einkorn (*T. monococcum*), and it is genetically described as a diploid with two sets of chromosomes (Feldman & Levy, 2023). At the same time, Emmer wheat (*Triticum dicoccum*) was also cultivated (Figure 1).

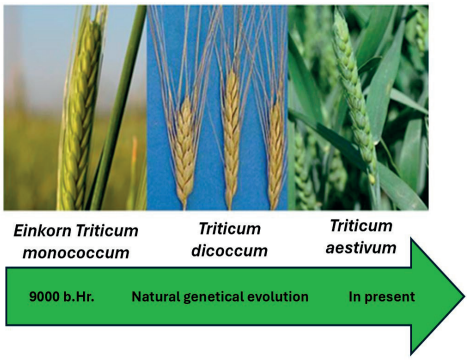


Figure 1. Wheat evolution (https://www.yara.hu/tapanyagellatas/oszi-buza/buza-tortenelmi-fejlodese/)

Winter wheat is one of the most demanding agricultural plants in terms of soil preparation. It can be said without reservation that the condition of the soil at sowing determines how the plants grow in autumn and their ability to easily get through the long winter period (Hassan et al., 2014).
The genetic evolution of wheat occurred through natural hybridization between the wild diploid species *Triticum urartu* (Wild Einkorn – *T. boeoticum*) and *Aegilops*, resulting in a new variety, a tetraploid, with four sets of chromosomes (Muhammad et al., 2023).
By cultivating this variety, farmers continued to select over the years from wheat crops that had better characteristics regarding yield and ease of harvesting.
They chose einkorn and common wheat, which were the results of natural hybridizations between Emmer wheat and the wild species *Aegilops tauschii*, which made the transition from a tetraploid to a hexaploid, which already contained six sets of chromosomes (42 chromosomes), and the yields were much higher than the original varieties, with 14 chromosomes (Mirzaghaderi, 2020).

Worldwide, wheat crops occupy 20% of the total cultivated area, according to current statistics. The latest USDA (US Department of Agriculture) report reported that the world wheat harvest in the 2023-2024 agricultural year was 789 million tons, being the most important cereal crop in the European Union and the most widespread food crop globally.
With high ecological plasticity, wheat is grown on all continents, between 45º South latitude and 66º North latitude, at an altitude from sea level to altitudes of over 3000 m.
According to recently updated statistics, the dynamics of wheat production at the level of the largest wheat producers, during the period 2016-2024 is highlighted in the Figure 2.

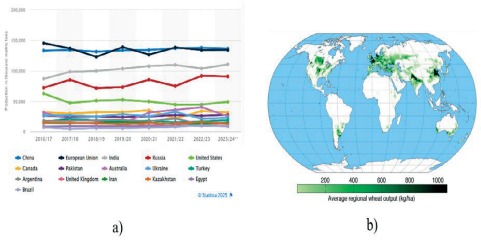


Figure 2. Wheat production (thousand tons) for the top ten producers, 2017-2024, according to Statista7, 2025 (a) and map of average global wheat production (b) (www.statista-com)

Shelterbelts have been used for centuries to improve microclimate and environmental conditions and increase crop yields (Cai, 2021).

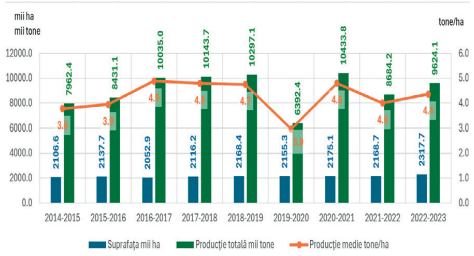


Figure 3. Graph regarding the dynamics of cultivated areas, total production and average wheat production obtained in Romania during the period 2014-2023

According to statistics carried out by the MADR Bucharest (Ministry of Agriculture and Rural Development of Romania), the Figure 3 represents the dynamics of wheat cultivated areas and the productions obtained in Romania, during the period 2014-2023, noting that the

highest average wheat production was 4.9 tons/ha in 2017, followed in descending order by 2018 and 2021 with 4.8 tons/ha, and 4.7 tons in 2019.
The lowest average production obtained was 3.0 tons/ha, in 2020, when the pedological drought was very extreme in most cultivated areas.

MATERIALS AND METHODS

Every agricultural year, at ARDS Braila, 25 wheat varieties and lines are tested in demonstration lots, along with other winter cereals, to choose the most performing genotypes adapted to the pedoclimatic conditions in interest and to provide recommendations for farmers.
The wheat varieties tested in the demonstration lots come from both Romanian and foreign germplasm, for comparison, in the classic technology of ARDS Braila.
During the two agricultural years 2022-2023 and 2023-2024, climate conditions were monitored throughout the winter wheat growing season, as well as field observations and biometric measurements, and at harvest, productivity indices were determined for all 25 varieties, compared to the average of the experience.
The experimental results were processed using the Anova test to determine the best performing wheat genotypes out of the 25 varieties tested in the two agricultural years.
Wheat varieties testing at SCDA Braila is carried out in comparative competition fields, to select the most performing genotypes in the plant breeding process.

RESULTS AND DISCUSSIONS

According to current statistics, in 2024, wheat was cultivated in Romania on an area of 2.18 million ha, and the average production per hectare was 4.99 t/ha (Figure 4).
The climatic data from the two agricultural years in which the tests took place are presented in Table 1 and Table 2.

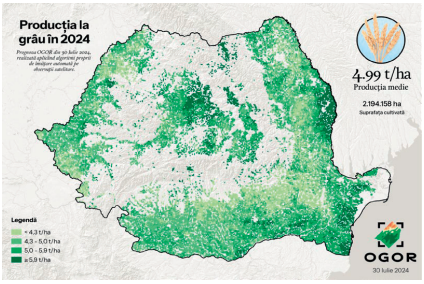


Figure 4. Map of wheat production in 2024 in different areas of Romania (www.ogor.ro)

Table 1. Climatic elements in agricultural year 2022-2023 at SCDA Braila

Climatic elements		2022				2023								TOTAL
		IX	X	XI	XII	I	II	III	IV	V	VI	VII	VIII	Average
Precipitation (mm)	Month average	32	6	31	20	64	7	13	66	40	26	106	55.1	466.1
	Multiannual month average	32	30	33	36	28	27	26	35	48	62	46	39	442
	Deviation	0	-24	-2	-16	36	-20	-13	31	-8	-36	60	16.1	24.1
Temperature (°C)	Month average	17.9	13	8.1	2.9	4.4	1.4	7.9	10.4	16.6	21.6	24.7	24.7	12.8
	Multiannual month average	17.3	11.5	5.6	0.6	-2.1	-0.2	4.7	11.2	16.7	20.9	22.9	22.1	10.9
	Deviation	0.6	1.5	2.5	2.3	6.5	1.6	3.2	-0.8	-0.1	0.7	1.8	2.6	1.9

Table 2. Climatic elements in agricultural year 2023-2024 at SCDA Braila

Climatic elements		2023				2024								TOTAL
		IX	X	XI	XII	I	II	III	IV	V	VI	VII	VIII	Average
Precipitation (mm)	Month average	4.5	3.4	124.0	22.87	15.9	0.3	40.4	26.1	35.0	47.3	33.2	17.2	370.1
	Multiannual month average	32	30	33	36	28	27	26	35	48	62	46	39	442
	Deviation	-27.5	-26.6	91.0	-13.1	-12.1	-26.7	14.4	-8.9	-13.0	-14.7	-12.8	-21.8	-71.9
Temperature (°C)	Month average	20.9	15.9	8.1	3.6	0.4	7.4	8.2	16.80	16.6	24.3	26.5	25.2	14.5
	Multiannual month average	17.3	11.5	5.6	0.6	-2.1	-0.2	4.7	11.2	16.7	20.9	22.9	22.1	10.9
	Deviation	3.6	4.4	2.5	3.0	2.5	7.6	3.5	5.6	-0.1	3.4	3.6	3.1	3.6

Compared to the agricultural year 2022-2023, when the deviation of the total recorded precipitation was + 24.1 mm compared to the multiannual, in the agricultural year 2023-2024 the precipitation deviation was -71.9 mm.
Regarding the average monthly temperatures recorded in the same analysed periods, deviations from the multiannual average of +1.9ºC were noted in the agricultural year 2022-2023 and +3.6ºC in the agricultural year 2023-2024.
In Figure 5 is represented an image with the experimental field from SCDA Braila.



Figure 5. Image from the experimental field with different genotypes of autumn cereals at SCDA Braila

Table 3 shows the production results of the 25 varieties tested in the agricultural year 2022-2023, and Figure 6 shows the production graph, compared to the average of the experiment.
The average o experience was 8439 kg/ha in 2023 and 9462 kg/ha in 2024, and these values represented the control for each experience.

Table 3. Production results and Anova test for the wheat varieties tested in year 2022-2023

Nr. crt.	Variants	Production kg/ha	Relative production %	Difference kg/ha	Difference signification
1	BIHARIA	10055	119	1616	***
2	Gabrio	9146	108	707	-
3	IZVOR	8984	106	545	-
4	URSITA	8958	106	519	-
5	PITAR	8941	106	502	-
6	FDL COLUMNA	8916	106	477	-
7	FDL ABUND	8744	104	305	-
8	ŞIMNIC S1412	8688	103	249	-
9	FDL EVIDENT	8620	102	181	-
10	VOINIC	8599	102	160	-
11	FDL DARNIC	8566	102	127	-
12	16286G3INC01	8529	101	90	-
13	FDL CONSECVENT	8494	101	55	-
14	OTILIA	8480	100	41	-
15	Average of experience	8439	100	0	Control
16	CARACAL 1	8368	99	-71	-
17	ŞIMNIC S1619	8336	99	-103	-
18	CARO	8269	98	-170	-
19	FDL EMISAR	8197	97	-242	-
20	DACIC	8135	96	-304	-
21	FDL ARMURA	8077	96	-362	-
22	GLOSA	7909	94	-530	-
23	FDL CONCURENT	7869	93	-570	-
24	MIRANDA	7842	93	-597	-
25	FDL AMURG	7617	90	-822	-
26	BEZOSTAIA	6629	79	-1810	○○○
	DL 5% = 872		DL 1% = 1182		DL 0,1% = 1583

The best results in demonstration plots of wheat varieties in agricultural year 2022-2023 were obtained by Biharia variety with +19% difference compared with control, and the lowest production result was obtained by the Bezostaia variety with a negative difference of -21% (Figure 6).

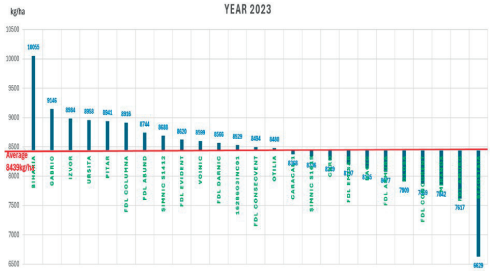


Figure 6. Production of wheat varieties tested in demonstration plots in year 2022-2023 at SCDA Braila

Table 4 shows the production results of the 25 varieties tested in the agricultural year 2023-2024, and Figure 7 shows the production graph, compared to the average of the experiment.

Table 4. Production results and Anova test for the wheat varieties tested in year 2023-2024

Nr. crt.	Variants	Production kg/ha	Relative production %	Difference kg/ha	Difference significance
1	FDL ABUND	10620	112	1158	***
2	ŞIMNIC S1412	10372	110	910	**
3	LOVRIN 9Z	10121	107	659	*
4	ŞIMNIC S1619	10083	107	621	*
5	FDL DARNIC	10033	106	571	*
6	FDL EVIDENT	9995	106	533	-
7	CEZAR	9876	104	414	-
8	A 4-10	9855	104	392	-
9	URSITA	9839	104	377	-
10	LOVRIN 90	9821	104	358	-
11	BIHARIA	9742	103	279	-
12	FDL AMURG	9691	102	229	-
13	DACIC	9633	102	171	-
14	OTILIA	9503	100	41	-
15	MIRANDA	9493	100	31	-
16	Average	9462	100	0	Control
17	FDL EMISAR	9431	100	-31	-
18	VOINIC	9383	99	-79	-
19	CARO	9100	96	-362	-
20	PITAR	9096	96	-366	-
21	GLOSA	9058	96	-404	-
22	FDL COLUMNA	8912	94	-550	0
23	IZVOR	8875	94	-587	0
24	FDL FAGUR	8735	92	-727	0
25	FDL	8041	85	-1422	000
26	BEZOSTAIA	7249	77	-2214	000
	DL 5% = 541kg/ha		DL 1% = 734 kg/ha		DL 0,1% = 983 kg/ha

The best results in demonstration plots of wheat varieties in agricultural year 2023-2024 were obtained by FDL Abund variety with +12% difference compared with control, followed by Simnic S1412 with +10%, and Lovrin 9Z and Simnic S1619, both with +7%, and the lowest production result was obtained by the Bezostaia variety with a negative difference of -23%, followed by FDL with -15%, FDL Fagur with -8%, Izvor and FDL Columna with -6% (Figure 7).

The Miranda variety stood out for its ability to adapt to drought climatic conditions, such as the year 2024, in which the precipitation deficit was -71.9 mm, and the average annual temperature had a deviation of +3.6ºC, compared to the year 2023, in which there was no precipitation deficit (+24.1 mm), and the deviation of the average annual temperature was +1.9ºC.

Thus, in the pedoclimatic conditions of 2023, compared to the average of the experiment, the Miranda variety obtained a production of 7842 kg/ha, with an insignificant negative difference compared to the average of the experiment, while in 2024, much drier than the previous year, the Miranda variety obtained a higher production compared to the control, respectively 9493 kg/ha (Figure 7).

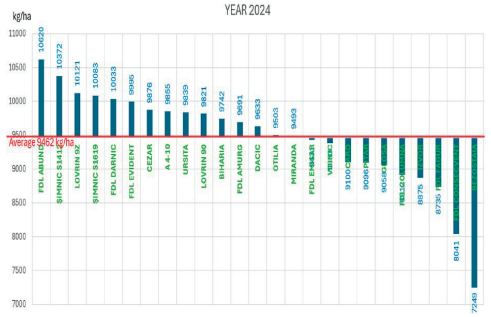


Figure 7. Production of wheat varieties tested in demonstration plots in year 2023-2024 at SCDA Braila

The best results regarding production indices in the wheat demonstration plots were obtained in 2023 by FDL Evident variety (51.31 g) followed by Glosa (50.05 g) and Voinic (47.90 g) for the mass of thousand seeds (Figure 8) and by Pitar variety (78.7 kg/hl) followed by FDL Abund (78.2 kg/hl) and Biharia (78.1 kg/hl) for hectoliter mass (Figure 9).

For the agricultural year 2023-2024, the best results for the mass of thousand seeds were obtained by Ursita variety (55.69 g), followed by Lovrin 90 (50.80 g) and Voinic (50.73 g) (Figure 10)

The best results for hectoliter mass were obtained by FDL Evident and Glosa varieties (83.0 kg/hl) and FDL Fagur (82.4 kg/hl) (Figure 11).

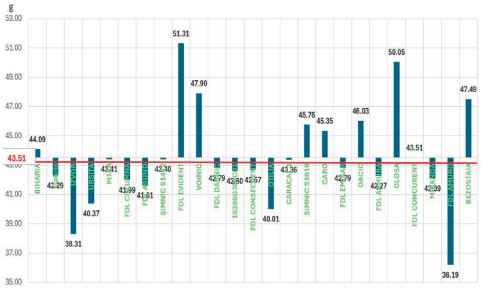


Figure 8. The mass of thousand seeds of wheat varieties tested in demonstration plots in year 2022-2023 at SCDA Braila

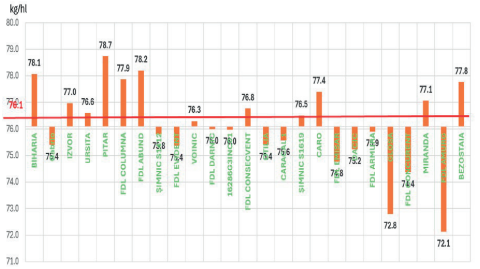


Figure 9. The hectoliter mass of wheat varieties tested in demonstration plots in year 2022-2023 at SCDA Braila

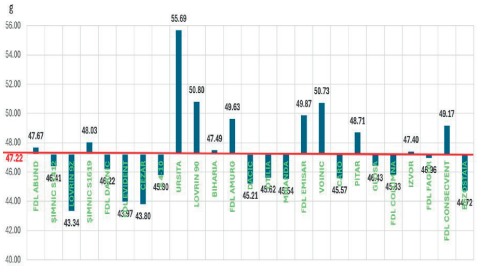


Figure 10. The mass of thousand seeds of wheat varieties tested in demonstration plots in year 2023-2024 at SCDA Braila



Figure 11. The hectoliter mass of wheat varieties tested in demonstration plots in year 2023-2024 at SCDA Braila

Being the most important cereal in human nutrition, wheat requires analysis and monitoring of quality indicators of harvested grains and research experiences to formulate a correct strategy for increasing the quantitative, but especially qualitative production of wheat crops. The most sought-after wheat varieties now are those with a high protein content, which increases the selling price of the production for the farmer producer.

The quantity and composition of protein substances (prolamins, glutens, etc.) determine the nutritional quality of the wheat grain, and their accumulation in the grains depends both on the genetics of the cultivated variety, but also on the pedoclimatic conditions, the fertilization practiced, and the treatments used.

Fertilizer application strategies and timely treatments to combat weeds, diseases and pests can increase the competitiveness and sustainability of wheat production, but genetic characteristics are the most important for competitive production.

Zoned wheat varieties are the most competitive because they already have the tolerance of the environmental conditions in which they were created and/or acclimatized.

To increase wheat productivity and the profitability of this crop, farmers must constantly adopt strategies to change and improve crop technology, by choosing the right zoned varieties, by applying fertilizers in stages, by applying phytosanitary treatments on time, to keep crops free of weeds, diseases, and pests.

Another very important thing for processing the resulting production in very good conditions is that the harvest is carried out on time, with a humidity close to STAS, and the transport and storage of the production is carried out in good, hygienic conditions in terms of temperature, humidity and non-infestation indices of the storage space.

CONCLUSIONS

- The demonstration plots organized at SCDA Braila with different wheat varieties are especially important for choosing the best genotypes from the breeder's seed for future multiplication, so that they are adapted to local soil and climatic conditions.
- Based on the results obtained in the last two agricultural years, the Abund wheat variety has already been chosen for multiplication, but demonstration batches will continue to be carried out with other new wheat genotypes.
- Cultivation technology is also of major importance, for ensuring adequate nutrition, as well as for preserving soil fertility, in the increasingly accentuated conditions of aridification and desertification, due to climate change and global warming.
- The Miranda variety is indicated in arid areas, having a similar production potential in dry years, compared to normal years in terms of precipitation.
- It is essential to explore solutions to reduce operational costs, diversify crops to have a market for the products obtained and invest in advanced technologies that can increase the efficiency and sustainability of agriculture.
- Regarding productivity indices, the best results were obtained by FDL Evident variety in 2023 and Ursita variety in 2024, for the mass of thousand seeds. The best results for hectoliter mass were obtained by Pitar variety in 2023, and FDL Evident and Glosa varieties in 2024.
- It is also crucial to support and revitalize the Romanian seed market to ensure better access to the most efficient seed material in the pedo-climatic conditions of the area.

ACKNOWLEDGEMENTS

This paper is an output of Doctoral School at the University of Agronomic Sciences and Veterinary Medicine of Bucharest, Doctoral Field: Agronomy.

This paper is an output of the science projects ADER 20.1.3, and ADER 1.2.2. financed by The Ministry of Agriculture and Rural Development, Contract 2013/17.07.2023, and 122/19.07.2023.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

HALOPHYTE BIOACTIVE COMPOUNDS: A REVIEW OF THEIR CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITIES

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Abstract

Halophyte plants, which thrive in saline environments (non-competitive agriculture areas), have garnered significant attention in recent years due to their potential as a rich source of bioactive compounds. These plants have evolved unique mechanisms to tolerate high salt concentrations, and in the process, they have accumulated a diverse array of secondary metabolites that are chemically and economically significant. Researchers have identified a wide range of bioactive compounds from halophytes, including phenolic compounds, terpenoids, alkaloids, and polysaccharides, each with their own distinct biological activities. These specialized metabolites often serve as osmoprotectants, antioxidants, and signalling molecules in the plants, conferring their characteristic resilience to high-salinity environments. This review synthesizes and examines the academic literature on secondary metabolites generated by halophyte plants.

Key words*: bioactive compounds, biological activities, halophytes, saline environments, secondary metabolites.*

INTRODUCTION

Natural products derived from secondary metabolites of plants play a crucial role in human and animal health, serving as primary sources of medicinal compounds (Qasim et al., 2017). These compounds are widely accepted due to their diversity, accessibility, sustainability, efficacy, and safety (Medini & Ksouri, 2018). Since ancient times, the study of plant-based bioactive compounds has been an essential part of drug discovery and development, enabling scientific validation of traditional remedies and the formulation of modern pharmaceuticals (Dangol, 2008; Kussmann et al., 2023).

Halophytes are plants adapted to saline conditions, and more than 2,500 genera of halophytes have been identified worldwide (Latruffe, 2010; Hameed et al., 2024). Many of these salt-tolerant species have potential as cash crops, with uses including livestock fodder, edible plants, medicinal resources, sources of chemical compounds, ornamental plants, and biofuel production (Sharma et al., 2016).

Saline environments, such as marine and hypersaline habitats, are widely distributed around the world. They include sea waters, saline lakes, solar salterns, or hypersaline soils (Vengosh, 2003: Torres et al., 2019). Primary salinity occurs naturally in soils and waters. Examples of naturally occurring saline areas include salt lakes, salt pans, salt marshes and salt flats. Secondary salinity is salting that results from human activities, usually land development and agriculture (Hassani et al., 2021). Water Mixing and Enclosed Seas: Limited freshwater mixing increases salinity. Examples: Black Sea, Caspian Sea, Red Sea, and Persian Gulf have high salinity (Beck et al., 2024).

Halophytes have been documented since the early 18th century, with their morphological and anatomical adaptations being closely linked to soil salinity and moisture levels (Toth et al., 2008; Panagos, 2022). Aquatic systems can be classified according their salinity as fresh (< 1 g L^{-1}), hyposaline (1-10 g L^{-1}), saline (10-100 g L^{-1}) and hypersaline (> 100 g L^{-1}) (Davis et al., 2003; Herbert et al., 2015). Although there is a lively discussion on a definition as exhaustive as possible, halophytes, in the broadest sense of the term, should be considered as all plant species occurring on a salty soil, recognized "macroscopically" by certain characters (xeromorphic aspects, flowering

saline, salt dust), but also by some indicator species of soils saline (Flowers & Colmer, 2008; Flowers & Muscolo, 2015; Santos et al., 2016).
Notable examples of edible halophytes and salt-tolerant species include: Marine fennel (*Crithmum maritimum* L.) (Lemoine et al., 2024); Mediterranean saltwort (*Soda inermis* Fourr.) (Agudelo et al., 2021); Glassworts (*Salicornia* spp.) (Nájar et al., 2023); Seablite (*Suaeda maritima*) (Alfheeaid et al., 2022). Several halophyte species are already utilized as food, forage, oilseed crops, and medicinal plants (Barreira et al., 2017). The significant diversity of saline habitats in Romania provides an optimal framework for investigating the chemical diversity and biological activities of halophytes (Ben Hsouna et al., 2022). The best-known species of halophytes in the maritime area of the Danube Delta, and not only, are: *Salicornia europaea, Suaeda maritima, Limonium gmelinii, Halimione Verrucifera*, *Festuca arundinacea, Portulaca oleracea, Gypsophila elegans, Ipomoea* (Grigore, 2008; Gavra, 2015).
Environmental factors, particularly those related to high salinity, arid climates, and nutrient scarcity, have highlighted the importance of halophytic plants in medicinal and agricultural sciences (Rozentsvet et al., 2021). The concept of halo-biotechnology the use of halophytes for agriculture and ecological restoration has emerged as a viable strategy for rehabilitating saline lands and establishing sustainable production systems (Musimba, 2012; Lombardi et al., 2023; Loconsole et al., 2019). Given the increasing pressure on freshwater resources, cultivating halophytes could provide an alternative agricultural model, but its success depends on economic viability, environmental sustainability, and the development of effective agronomic practices (Buhmann & Jutta, 2013; Saleem et al., 2021).
These plants have evolved unique adaptive mechanisms to survive under extreme conditions, producing a higher concentration of secondary metabolites such as phenolics, flavonoids, alkaloids, and saponins (Hulkko et al., 2022; Hulkko et al., 2023) These compounds contribute to the plants' defense mechanisms against oxidative stress, microbial infections, and herbivory while also offering significant nutritional and medicinal benefits (Todorović et al., 2022).
Certain halophytic plants from other areas, including *Lycium shawii, Anabasis articulata, Zilla spinosa,* and *Rumex vesicari*us, have demonstrated remarkable adaptability to these harsh conditions. Their ability to thrive in saline environments is attributed to specialized physiological and biochemical responses, such as the production of enzymatic and non-enzymatic antioxidants to counteract oxidative stress. These edible halophytes exhibit a nutritional profile suitable for human consumption. They contain high levels of protein (5.20-13.2 g per 100 g dry weight) and are rich in n-3 polyunsaturated fatty acids (omega-3 fatty acids), particularly α-linolenic acid, which accounts for about 19.3-25.9% of the total fatty acids. Furthermore, the concentrations of toxic metals in these plants are low, remaining below the limits established by the European Commission for food safety. These plants are traditionally used in various medicinal and nutritional applications (Ivanova et al., 2009). For instance, *Lycium shawii* has been historically employed for infection control, *Rumex vesicarius* as a diuretic and treatment for gastrointestinal disorders, *Anabasis articulata* for diabetes management, and *Zilla spinosa* for urinary and gallbladder ailments.
Globally, soil and water salinization pose significant threats to agricultural productivity, affecting nearly 1 billion hectares of land. Halophytes, defined as plants capable of completing their life cycle in high-salinity conditions, have evolved various tolerance mechanisms, distinguishing them from glycophytes, which lack such resilience. Adaptations to saline environments involve morphological, physiological, and biochemical modifications that allow halophytes to manage osmotic stress, ion toxicity, and nutrient imbalances (Hameed et al., 2010). These adaptations include salt secretion, succulence, ion sequestration, and specialized transport mechanisms (Ashraf et al., 2010).
This work reviews recent findings on halophytes under saline conditions, highlighting their ecological and agricultural potential, as well as the challenges and considerations necessary for their large-scale application.

MATERIALS AND METHODS

The searches for this review were conducted in the databases Scopus, Web of Science, SciFinder and Google Scholar. Articles published from the year 1995 till present were used for the review with some inclusion and exclusion criteria. The search terms included (for example) “hypolipidemic and hepatoprotective screening of halophytes species”, “hypolipidemic and hepatoprotective analysis of halophytes species”, “halophytes species with hypolipidemic and hepatoprotective activity”, and “hypolipidemic and hepatoprotective activity of bioactive compounds from halophytes species”. Major references from some of the articles were also searched for additional information.

Based on the number of articles accessed, some inclusion and exclusion criteria were applied, so as to include the most relevant articles in this review. Publications that extensively examined the efficacy of the bioactive compounds of halophytes species were included (Zakirova et al., 2024; Piatti et al., 2021; Purushothaman et al., 2024). I have kept mostly references from recent years, because halophytes have not been scientifically investigated and analyzed for too long time.

CHARACTERISTICS OF HALOPHYTIC PLANTS

Increasing salinization has severe consequences for land, water, vegetation, wildlife, and agricultural productivity, particularly in arid and semi-arid regions where high evapotranspiration rates intensify environmental stress (Shahid et al., 2020). Unlike conventional crops, halophytes salt-tolerant plants found in coastal, wetland, and desert environments have evolved sophisticated adaptive mechanisms at the whole-plant, cellular, and molecular levels, enabling them to thrive under high salinity (Ghanem et al., 2021). Representing approximately 1% of the world's flora, these species offer a promising solution for utilizing the estimated one billion hectares of salt-affected land worldwide.

To be widely adopted, saline agriculture must ensure sufficient crop yields and profitability while preventing further environmental degradation (Karkanis et al., 2022). Halophyte domestication holds potential for producing food, forage, oilseeds, pharmaceuticals, and ornamental plants. Additionally, certain halophytes could contribute to soil desalination, enhancing the productivity of marginal lands. However, the effective implementation of this approach requires comprehensive research on the salt tolerance thresholds of candidate species throughout their life cycle.

Although, their common xeromorphic characteristics must be universally acknowledged, as they underpin their ability to thrive in saline environments. So, many halophytes are also consumed for their organoleptic (sensory) qualities and medicinal properties (Barreira et al., 2017). To date, over 7,000 plant species worldwide have been documented as edible (Shikov et al., 2017). However, only a very small fraction of these edible species are strict halophytes.

1. Taxonomic and Phylogenetic Considerations

Halophytes belong to diverse plant families, with representatives across multiple taxonomic groups. Notable families that include halophytic species are: *Acanthaceae, Aizoaceae, Amaranthaceae, Apiaceae, Asteraceae, Brassicaceae, Caryophyllaceae, Combretaceae, Convolvulaceae, Euphorbiaceae, Fabaceae, Frankeniaceae, Fumariaceae, Juncaceae, Lamiaceae, Malvaceae, Plantaginaceae, Plumbaginaceae, Primulaceae, Rhizophoraceae, Salvadoraceae, Salicornia*, *Tamaricaceae,* and *Zygophyllaceae* (Shahid et al., 2020).

Phylogenetic studies have revealed that halophytism has evolved multiple times across plant lineages, with distinct strategies emerging in different ecological niches. Understanding these evolutionary pathways may aid in identifying genetic determinants of salt tolerance and developing salt-resistant crops (Cárdenas-Pérez et al., 2021).

2. Adaptation Mechanisms of Halophytes

Halophytes, particularly those from the *Chenopodiaceae* family, exhibit diverse strategies for coping with salinity, such as accumulating or secreting salts. Their ability to grow optimally in saline conditions makes them valuable for ecological and agricultural

applications. Studying their elemental composition and phylogenetic relationships provides insights into their adaptive mechanisms and potential uses (Ashraf et al., (2010).
Aspects and problems related to the adaptive mechanisms of halophytes will be analysed in a future paper.

3. Compounds and secondary metabolites

Halophytes possess adaptive mechanisms that enable them to retain and absorb water, protect cellular structures from oxidative stress induced by reactive oxygen species, and maintain ion homeostasis in saline environments. These adaptations involve the biosynthesis of various bioactive compounds with significant biological properties, including antioxidant, antimicrobial, anti-inflammatory, and antitumoral activities. Consequently, halophytes have potential applications in disease prevention, particularly in reducing the risks of cancer, chronic inflammation, and cardiovascular disorders when incorporated into the human diet (Fakhri et al., 2021).
Moreover, these bioactive compounds contribute to the enhanced nutraceutical value of halophytic grasses, as their concentration and diversity are typically higher than those found in non-salt-tolerant crop species (Kraouia et al., 2023). Certain specialized metabolites are exclusive to halophytes, offering promising applications in the agri-food, pharmaceutical, and cosmetic industries (Correia et al., 2022). These unique properties underscore the importance of halophytes as valuable resources for both human health and industrial applications.
The complexity of halophyte adaptation is reflected in their biochemical composition. These plants synthesize a wide array of secondary metabolites with significant ecological and pharmacological relevance (Rodrigues et al., 2018). Some of the most common secondary metabolites in halophytes include:
- ***Phenolic compounds and flavonoids***. Flavonoids are classified as flavone or isoflavones based on the position of the benzoid ring. Phenolic compounds have been widely reviewed and reported to have beneficial effects on human health such as anticancer, antimicrobial, and antimutagenic properties. Phenolic acids such as vanillic, caffeic, ferulic and protocatechuic can be present in some halophytes (Lopes et al., 2021; Lopes et al., 2023; Srivarathan et al., 2023)
- ***Saponins and alkaloids***. Play protective roles against herbivores and pathogens, while also exhibiting medicinal properties (Medini & Ksouri (2018).
- ***Triterpenes and sterols***. Provide structural integrity to cell membranes and enhance stress tolerance.
- ***Osmoprotectants***. Such as betaines and proline, which help maintain cellular function under salt stress conditions.

Halophyte secondary metabolism is particularly significant from a pharmaceutical perspective, as many of these bioactive compounds have demonstrated antimicrobial, anti-inflammatory, hepatoprotective, and antidiabetic properties.

4. Ecological and Agricultural Significance

Halophytes hold great potential for ecological restoration and sustainable agriculture. Their ability to thrive in saline environments makes them candidates for:
- ***Soil remediation***. Certain species, such as *Salsola* and *Salicornia*, can absorb and sequester salts, improving soil quality in degraded coastal and inland regions (Castagna et al., 2022).
- ***Alternative crops***. Some halophytes can be cultivated as food or fodder crops in saline soils where conventional crops fail to grow (Kraouia et al., 2023).
- ***Medicinal applications***. Due to their bioactive compounds, these plants are of interest for drug discovery and development (Fangjie et al., 2024).

Halophytic plants represent a unique ecological group characterized by their specialized adaptations to saline environments (Clavel-Coibrié et al., 2021). Their physiological mechanisms, phytochemical diversity, and ecological roles make them valuable for scientific research, conservation, and agricultural innovation (Alhaddad et al., 2021). Continued exploration of halophyte biology will enhance our understanding of plant stress tolerance and open new avenues for sustainable resource utilization in saline-prone ecosystems.
It is concluded that halophytes may become important sources of natural products for the treatment of various ailments and for

supplementing the human diet with necessary non-nutrients and minerals. However, extensive studies are needed to deepen the knowledge of their biological potential in vivo, so that they can be introduced to the pharmaceutical and food industries.

PHYTOCHEMICAL DIVERSITY OF HALOPHYTES

Studies have identified a diverse array of bioactive compounds in halophytes, including alkanes, alkenes, fatty acids, acylglycerols, cinnamic acids, benzoic acids, short-chain carboxylic acids, carbohydrates, amino acids, alcohols, aldehydes, ketones, terpenoids, tocopherols, flavonoids, polyphenols, alkaloids, stilbenoids, and their derivatives, along with various other specialized metabolites (Kim et al., 2021; Lopes et al., 2021; Lopes et al., 2023; Srivarathan et al., 2023).

These compounds contribute to the functional and pharmacological properties of halophytes (Srivarathan et al., 2023), further reinforcing their potential applications in medicinal, nutritional, and industrial domains (Roberto et al., 2021).

Bioactive compounds of Halophytes (Rahmanie et al., 2022; Markovskaya et al., 2024):

- hydrocarbons;
- organic acids, acylglycerols and derivatives;
- strong-smelling isoprenoids;
- polyphenols;
- hydrocarbon chains with c6-c2-c6 structure; derivatives of stilbene;
- other compounds.

BIOLOGICAL ACTIVITIES OF HALOPHYTES

Plant adaptation and phytoremediation (Ashraf et al., 2010), exhibits the highest diversity. To emphasize, various halophyte species have been used in folklore medicine and increasing number of modern chemical and pharmacological reports have revealed that halophyte plants have many beneficial effects, including nutraceutical value, anti-diabetic and anti-obesity activities, anti-oxidant (Saleh et al., 2021) neuroprotective (Khaled et al., 2024), anti-Alzheimer, anti-ulcer (Jaiswal et al., 2024), anticarcinogenic activity (Custodio et al., 2022)., anti-viral/antifungal (Fredsgaard et al., 2023), antibacterial (Al-Judaibi, 2020), anti-hypertensive, hypolipidemic and hepatoprotective (Souid, 2020), hypoglycemic, anti-inflammatory effects (Karker et al., 2023) spasmolytic and antidiarrheal activities (Chen Hsiao-Ting et al., 2023)

Moreover, halophytic plants represent a potential reservoir of edible and highly nutritious species, which is particularly relevant in the present context. Over the past decade, the global population has continued to grow, while the availability of arable land has steadily declined due to increasing soil salinity (Todorović et al., 2022). It is estimated that approximately 1,000 million hectares of land are affected by this phenomenon, accounting for 20% of the world's cultivated areas. Soil salinity is widely recognized as a significant threat to global food security and agricultural sustainability. However, certain highly salt-tolerant species within the Poaceae family offer a promising solution, as they can withstand seawater-level salt concentrations while also exhibiting substantial nutraceutical potential. Notably, some of these species have already been deemed suitable for livestock consumption, as exemplified by *Beckmannia syzigachne* (Steud.) (Faustino et al., 2019).

FUTURE CHALLENGES ON THE RESEARCH

Furthermore, halophytic plants represent a valuable reservoir of highly nutritious and edible plant species, an essential consideration given the increasing global population and the continuous decline in arable land due to soil salinization. Currently, an estimated 1000 million hectares of land, accounting for approximately 20% of the world’s cultivated area, are affected by salinity. This presents a major challenge to global food security and agricultural sustainability. However, certain salt-tolerant species offer a promising solution, as they are capable of surviving in seawater-level salinity while also exhibiting high nutraceutical potential (Alfheeaid et al., 2022). Some of these species, such as *Beckmannia syzigachne* (Steud.), are already recognized as valuable fodder crops for livestock, further emphasizing the potential role of halophytes in

future agricultural systems aimed at addressing food security challenges.

In addition, halophytic plants could be a reservoir of edible and highly nutritional plants which is vital today, since in the past decade, the world population has increased continuously while a constant reduction of arable lands is observed due to increased soil salinity (Jia et al., 2011). It is estimated that 1000 million hectares of land are affected by this issue, which corresponds to 20% of the world-cultivated area (Kafi & Khan, 2008). Soil salinity is considered a serious threat to global food security and sustainability; however, a glimmer of hope lies on the existence of some truly salt-tolerant plants from *Poaceae*, which can survive in seawater salt concentrations and simultaneously have high nutraceutical potential. For instance, some of these species have already been considered edible for cattle, which is the case of *Beckmannia syzigachne* (Steud.), *Cenchrus ciliaris* L., *Echinochloa colona* (L.) Link (Dangol, 2008). *Echinochloa crus-galli* (L.), *Dactyloctenium aegyptium* (L.) Willd. (Dedrilkumar & Binu, 2016), *Imperata cylindrica* (L.) Raeusch. (Subramaniam & Sivasubramanian, 2015), *Leymus arenarius* (L.) Hochst. (Svanberg & Egisson, 2012), *Phragmites australis* (Cav.) Trin. ex Steud. (Agudelo et al., 2021) and *Zizania aquatica* L. (Zhang et al., 2014).

RESULTS AND DISCUSSIONS

Halophytes possess adaptive mechanisms that enable them to retain and absorb water, protect cellular structures from oxidative stress induced by reactive oxygen species, and maintain ion homeostasis in saline environments.

These adaptations involve the biosynthesis of various bioactive compounds with significant biological properties, including antioxidant, antimicrobial, anti-inflammatory, and antitumoral activities (Nasernakhaei, 2021; Renna & Gonnella, 2021; Limongelli et al., 2022; Li et al., 2024). Consequently, halophytes have potential applications in disease prevention, particularly in reducing the risks of cancer, chronic inflammation, and cardiovascular disorders when incorporated into the human diet.

Review of the bioactive compounds in halophytes reveals an impressive chemical diversity, demonstrating the importance of these plants in traditional medicine and modern pharmaceutical research (Nazish et al., 2020). Halophytes contain compounds such as flavonoids, saponins, phenolic compounds and fatty acids, each with specific biological activities ranging from antioxidant and anti-inflammatory effects to antimicrobial and antidiabetic properties (Gourguillon et al., 2016; Gheraissa et al., 2024).

For example, *Salicornia herbacea*, analyzed in detail by Rhee & Park (2009) contains phenolic acids such as tungtungmadic acid (*quinic acid*) and flavonoids such as quercetin 3-O-glucoside and isorhamnetin 3-O-glucoside. These substances have been found to have potent antioxidant and anti-inflammatory properties, justifying the traditional use of the plant in the treatment of conditions such as diabetes and atherosclerosis (Arya Sunder Singh et al., 2019). On the other hand, *Polygonum maritimum* L. (sea knotgrass/knotweed), another halophyte of pharmaceutical interest (Rodrigues et al., 2018), has been studied for its effects on salt stress and secondary metabolite content (Oliveira et al., 2023). Studies on this plant have shown that salinity levels significantly influence its chemical composition, affecting antioxidant and anti-inflammatory activities (Rodrigues et al., 2018). This underlines the importance of environmental factors in the biosynthesis of bioactive compounds and thus in the therapeutic potential of halophytic plants

Chemically, halophytes produce a broad range of secondary metabolites of economic significance (Katel et al., 2023). Several of these compounds are unique to halophytic species or exist in higher concentrations compared to glycophytes. For their optimal exploitation, precise plant cultivation conditions, as well as efficient extraction, fractionation, and isolation processes, must be established. While individual bioactive compounds can be specifically identified and controlled, crude extracts containing a mixture of these compounds may exhibit synergistic effects, enhancing their functional potential. Additionally, the concentration and composition of secondary metabolites vary significantly among different

plant organs and across developmental stages (Kim et al., 2021).

The secondary metabolites derived from halophytes have potential applications in diverse fields, including pharmacognosy, functional foods, Halophytes possess adaptive mechanisms that enable them to retain and absorb water, protect cellular structures from oxidative stress induced by reactive oxygen species (ROS), and maintain ion homeostasis in saline environments, and industrial applications. While many of these applications remain in the research and development phase, certain halophyte-derived products have already reached commercial markets. Notable examples include species such as *Salicornia spp.* and *Crithmum maritimum*, which contain valuable compounds utilized in various industries (Melo et al., 2024). These attributes highlight the economic potential of halophytes and underscore their relevance for sustainable industrial and pharmaceutical applications (Ozturk et al., 2023).

CONCLUSIONS

Halophytes represent a specialized group of plants capable of thriving in saline environments where glycophytes fail to survive. These plants have evolved specific physiological and biochemical adaptations that enable them to endure high salt concentrations in diverse habitats, including seawater, salt marshes, and salt deserts. Nearly all halophytes are angiosperms, with dicotyledonous species often exhibiting growth stimulation in response to moderate salinity, while monocotyledonous species generally do not display this trait.

Given that a substantial portion of global water resources is saline, the agricultural importance of halophytes is increasingly recognized, particularly in the context of food security and sustainable land use. Halophytes have undergone significant evolutionary adaptations, allowing them to tolerate high salinity levels due to specialized traits developed over time. These plants exhibit a broad range of halotolerance, making them valuable models for studying salt tolerance mechanisms. Although halophytes constitute only about 2% of terrestrial plant species, they are represented across nearly half of all higher plant families, demonstrating remarkable diversity in their life forms.

In saline environments halophytes must address two primary challenges: tolerating high soil salinity and absorbing water from a medium with low water potential (Ivanishev, 2021). To overcome these challenges, halophytes employ a variety of mechanisms, including the regulation of osmotic balance and ion transport. The increasing global prevalence of soil salinization, driven by factors such as excessive irrigation, industrial waste disposal, and groundwater contamination, poses a significant challenge to agricultural sustainability. Current estimates suggest that approximately 400 million hectares of land are affected by salinity, necessitating the development of salt-resistant crops to ensure food security. Genetic approaches aimed at enhancing salt tolerance in crops have shown promising results, with research focusing on the identification of key stress-tolerance genes and regulatory promoters. Given the rapid growth of the global population and the declining availability of arable land, the study of halophytes has become increasingly relevant. These plants hold potential applications in agriculture, animal fodder production, and medicinal research. Furthermore, halophytes are emerging as a viable alternative for biofuel production, providing a sustainable source of energy while mitigating the negative impacts of fossil fuel consumption (Sudhanshu & Ramachandran, 2021). By leveraging their genetic traits, researchers aim to develop transgenic crops with enhanced salt tolerance, paving the way for innovative agricultural solutions in saline-affected regions (Dagar & Gupta, 2024).

This review synthesizes existing knowledge on halophytes, integrating information from various research studies to provide a comprehensive understanding of their classification, chemical diversity and biological activities of their, and ecological significance.

This work aims to present a holistic overview, offering insights into their potential applications in sustainable agriculture and biotechnology, and more (Grigore et al., 2025).

The continued study of halophytes is imperative for addressing global challenges related to salinity, food security, and environmental conservation.

ACKNOWLEDGEMENTS

This work was carried out with the support of Ministry of Research, Innovation, and Digitization and financed from the Core Program (project 7N/23-02-0101/2023)

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

RECYCLING OF WASTEWATER FROM THE CULTIVATION OF *Spirulina platensis* THROUGH ITS USE AS A BIOSTIMULANT FOR THE GERMINATION OF *Phacelia tanacetifolia* Benth. (Melifera) SEEDS MAINTAINED IN COLLECTIONS

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Abstract

This article presents the experimental results obtained from the application of a biostimulant based on wastewater derived from the cultivation of the cyanobacterium Spirulina platensis in various concentrations, on the germination of Phacelia tanacetifolia (Melifera) seeds, maintained under collection conditions for 1 and 4 years. The results show that the seeds treated with biostimulants have a higher germination capacity compared to those in the control group, where germination ranged from 28-40%. The highest germination rates were obtained for the 1-year-old seeds (56%) treated for 1 hour with a biostimulant at a 2% concentration, while for the 4-year-old seeds (54%), the best results were recorded at a 1% concentration with a 1-hour treatment period. The application of the biostimulant contributed to the relative elongation of the root system of the studied culture. Based on the conducted research, we can conclude that the application of the investigated biostimulant has the effect of stimulating seed germination and root elongation in seedlings, which provides grounds to consider that it demonstrates a positive effect and could be practically applied.

Key words*: wastewater, biostimulator, Spirulina platensis, Phacelia tanacetifolia (Melifera).*

INTRODUCTION

The cyanobacteria *Spirulina platensis* is one of the most effective and applicable species in agriculture due to the high content of biologically active substances with a biostimulatory effect, the high growth rate and the possibilities of industrial cultivation. Spirulina can be applied as a biostimulator in the cultivation of crops and medicinal plants starting with the vegetative stage and until the end of the generative stage, resulting in positive effects both quantitatively and qualitatively.

Research conducted by Nguyen Quang Thinh highlighted the fact that treating *Vigna mungo* L. seeds before sowing, for 3 hours, with extract obtained from *Spirulina platensis* biomass at a concentration of 1.5%, highlighted the fact that it contributed to increasing the gemination rate by 17%, root elongation by 1.48 cm, shoot length by 1.53 cm, dry matter by 13.10 mg/10 seedlings, increasing the content of proteins, gibberellic acids, α-amylase, dehydrogenase activity accompanied by a decrease in lipid peroxidation and free sugar (Thinh, 2021).

Other research has demonstrated that treating Chilli, Tomato, Carrot, Bean, Maize, Paddy and Ragi seeds, for 12 hours, with *Spirulina platensis* biomass suspended in distilled water in various concentrations had the effect of stimulating seed germination, increasing seedling vigor, creating premises for crop growth and productivity (Basavaraja et al., 2023).

Other research has highlighted the fact that applying extracts obtained from *Spirulina platensis* biomass to the treatment of radish seeds has the effect of stimulating seed germination, increasing plant length and weight, chlorophyll, and increasing the content of nutrients in seedlings (Godlewska et al., 2019).

Treating chickpea seeds (*Cicer arietinum* L.*)* with *Spirulina* biomass at doses of 2%, 4%, 6%, 8% and 10% had the effect of stimulating seed germination, shoot growth and increasing protein content (Htwe et al., 2009).

Treating onion seeds before sowing with *Spirulina* sp. extract had the effect of increasing the germination rate and germination index (Neag et al., 2022).

The application of *Spirulina platensis* extracts in optimal concentrations of 25-75%, when treating wheat and barley seeds before sowing, had the effect of stimulating germination and seedling growth and development (Akgül, 2019).

Treating *Lactuca sativa* L. seeds before sowing with *Spirulina platensis* extract in optimal concentrations, also attested a positive effect on seed germination and plant growth-development (Fusun & Riza, 2019).

A major problem resulting from the cultivation process of *Spirulina platensis* is the wastewater that represents around 98-99.50% of the total volume of the suspension, these waters contain significant amounts of biologically active substances (micro and macroelements, metabolites, hormones, etc.) and are of interest for recycling as a biostimulator of plant seed germination.

Phacelia tanacetifolia is a plant species that is part of the *Hydrophyllaceae* family originating from the Americas, being brought to Europe in the last century and acclimatized for use as a fodder and pollinator plant, being increasingly used as a component of the cover crop in agriculture (Kilian, 2016). The plant is part of the top 20 flowers producing high-quality pollen for bees, being attractive to pollinating insects including bumblebees (Hayden, 2014). A plant has more than 5000 flowers and 500-1000 kg of honey is obtained from one hectare of *Phacelia tanacetifolia.* Phacelia has a positive impact on the soil due to the penetration of the crop's roots to depths of over 1 m, being considered a "green plow", and after harvesting the crop, the root residues accumulate significant amounts of organic matter and nitrogen, being considered an "organic fertilizer" and presenting considerable interest as a sidereal crop. Some research indicates that the *Phacelia tanacetifolia* variety has a yield of 25.000 kg/ha green mass and over 7.000 kg/ha dry mass (Popović et al., 2016).

To ensure high growth and obtain large amounts of biomass, it is necessary to treat the seeds preventively. Thus, we aimed to evaluate the impact of the biostimulator obtained from the wastewater generated from the cultivation of the cyanobacterium *Spirulina platensis* on the germination process of *Phacelia tanacetifolia* Benth. (Melifera) seeds.

MATERIALS AND METHODS

The experiments used the strain of cyanobacterium *Spirulina platensis* selected in culture by m.c., prof. Vasile Șalaru, acad., prof. Valeriu Rudic and co-authors. The strain of *Spirulina platensis,* stored in the collection of LCȘ "Algologie Vasile Șalaru" of the Moldova State University, was cultivated on modified Zarrouk liquid nutrient medium (with the following composition (g/l): $NaHCO_3$ - 8; K_3HPO_4 - 0.5; $NaNO_3$ - 2.5; K_2SO_4 - 1; NaCl - 1; $MgSO_4$ - 0.2; $CaCl_2$ - 0.04; $FeSO_4$ - 0.01; NaEDTA - 0.08; drinking water - 1 L), the inoculum constituting 0.5 g/l (absolutely dry biomass). On the 20th day of cultivation of the cyanobacterium *Spirulina platensis,* the algal biomass was separated from the culture liquid by centrifugation (at a speed of 6000 rpm), and the culture liquid (waste water) was used to obtain the biostimulant applied in the experiments. The cyanobacterial biostimulant was obtained by thermal activation of the separated culture liquid.

The experiments used solutions with concentrations of 1-4% obtained by diluting the culture liquid generated from the cultivation of the cyanobacterium *Spirulina platensis* with distilled water. Seeds of *Phacelia tanacetifolia* Benth. (Melifera), maintained in collection conditions for 1-4 years, in the amount of 100 for each experimental batch, were exposed to the prepared solutions for 1-4 hours, and as a control the same seeds exposed to distilled water for the same time interval served. The experimental seeds of *Phacelia tanacetifolia* Benth. (Melifera) were made available by Dr. Victor Ţiţei, an employee of the Botanical Garden of the Al. Ciubotaru Institute of the State University of Moldova. To study the germination process, the seeds were placed in Petri dishes, on filter paper moistened with distilled water, under natural lighting and a temperature of 22°C. The following indicators were determined during the research:

- *Seed germination* (FG), according to the formula: FG = Nsg/Nts * 100, where Nsg - number of germinated seeds; Nts - total number of seeds;
- *Germination index* (IG), calculated on the 5th day of the experiment, according to the formula: $IG = \sum(Gt/Tt)$, where Gt - number of germinated seeds in the time period t, and Tt - number of days.
- *Relative root elongation* (ARR) calculated on the 7th day of germination, according to the

formula: ARR = (Le/Lc)*100, where: Le - root length in the experimental variant; Lc - root length in the control variant.

RESULTS AND DISCUSSIONS

It is well known that treating plant seeds before sowing increases the biological value of these seeds, plant resistance to diseases and crop yield (Siminel, 1999). Taking into account the fact that the cyanobacterium *Spirulina platensis* has a major content of biostimulating substances for seed germination and that a good part of them are found in the culture liquid, this liquid is of major interest for use as a bistimulator for seed germination of *Phacelia tanacetifolia* Benth.

Table 1. Germination capacity of *Phacelia tanacetifolia* Benth. (Melifera) seeds treated with biostimulator obtained from the culture liquid resulting from the cultivation of the cyanobacterium *Spirulina platensis*, %

Seed treatment period, hours	Experimental variants							
	1%		2%		4%		Control	
	1 - year	4 - years	1 - year	4 - years	1 - year	4 - years	1 - year	4 - years
1- a day								
1	4.0	36.0	18.0	34.0	4.0	32.0	6.0	22.0
2	6.0	36.0	6.0	38.0	18.0	20.0	4.0	16.0
4	8.0	28.0	10.0	36.0	4.0	30.0	6.0	24.0
2- a day								
1	30.0	36.0	46.0	34.0	38.0	32.0	14.0	22.0
2	28.0	36.0	32.0	38.0	40.0	20.0	26.0	16.0
4	34.0	28.0	32.0	36.0	34.0	30.0	36.0	24.0
3- a day								
1	60.0	38.0	56.0	46.0	44.0	44.0	34.0	32.0
2	32.0	50.0	38.0	44.0	40.0	28.0	32.0	28.0
4	34.0	40.0	38.0	42.0	44.0	36.0	36.0	24.0
4- a day								
1	60.0	40.0	56.0	46.0	48.0	44.0	40.0	32.0
2	40.0	54.0	42.0	44.0	44.0	30.0	36.0	28.0
4	42.0	40.0	40.0	46.0	46.0	38.0	41.0	34.0

The results of the analysis of the germination capacity of *Phacelia tanacetifolia* Benth. (Melifera) seeds indicate that the biostimulator obtained from the wastewater generated from the cultivation of the cyanobacterium Spirulina platensis has a stimulating effect on the germination of the analyzed seeds. The most significant results were obtained in the variants of 1-2% biostimulator with exposure to the treatment for 1 hour in the case of seeds maintained in collections for 1 year where 60-56% of all analyzed seeds germinated. Keeping *Phacelia tanacetifolia* Benth. (Melifera) seeds in collection conditions for 4 years has the effect of reducing their germination capacity. In this case too, the treatment of the seeds with the investigated biostimulator resulted in the germination of a larger number of seeds (up to 54%) and in the maximum control variant 34% of the total analyzed seeds germinated. In the case of older seeds, as in the case of newer ones, the most suitable variant proved to be 1% with exposure to the treatment for 2 hours (Table 1). *Spirulina* is rich in metabolites with bioregulatory functions (Mehmood et al., 2024). A good part of the metabolites of the cyanobacterium *S. platensis* are released into the cultivation medium, which gives it a pronounced biostimulatory effect on the germination of *Phacelia tanacetifolia* Benth. (Melifera) seeds investigated by us.

Table 2. Germination index values of *Phacelia tanacetifolia* Benth. (Melifera) seeds treated with biostimulator obtained from the culture liquid resulting from the cultivation of the cyanobacterium *Spirulina platensis*

Seed treatment period, hours	**Experimental variants**							
	1%		**2%**		**4%**		**Control**	
	1 - year	**4 - years**	**1 - year**	**4 - years**	**1 - year**	**4 - years**	**1 - year**	**4 - years**
1	11.2	8.0	9.2	9.2	9.6	8.8	8.0	6.4
2	8.0	10.8	8.4	8.8	8.8	6.0	7.2	5.6
4	8.4	8.0	8.0	9.2	9.2	7.6	8.2	6.8

The germination index values of *Phacelia tanacetifolia* Benth. (Melifera) seeds also indicate that treating the seeds with the biostimulator studied has positive effects. The highest values were observed in the variants of 1-2% biostimulator with exposure to the treatment for 1 hour. The seeds of *Phacelia tanacetifolia* Benth. (Melifera) treated with the biostimulator studied showed much higher germination index values compared to those in the control variant (Table 2). Similar results were also obtained in the case of treating the seeds of *Galega orientalis* L. maintained under collection conditions with the cultural liquid generated from the cultivation of the cyanobacterium *Spirulina platensis* (Dobrojan et at., 2024) (Table 3).

Table 3. Relative root elongation of *Phacelia tanacetifolia* Benth. (Melifera) treated with biostimulator from the culture liquid of the cyanobacterium *Spirulina platensis*, %

Seed treatment period, hours	Experimental variants					
	1%		2%		4%	
	1 - year	4 - years	1 - year	4 - years	1 - year	4 - years
1	127.45	94.94	152.94	84.81	141.17	82.28
2	78.92	73.08	99.21	82.05	105.97	101.28
4	72.34	134.32	87.23	100.0	102.13	126.86

The highest values of relative root elongation were attested in the case of young 1-year-old seeds with exposure to the treatment for 1 hour in the treatment variant with 2% biostimulant (152.94%), followed by the concentration of 4% (141.17%) and 1% (127.45%). In the case of older seeds, the highest results of this indicator were attested in the variant with 4% exposure to the treatment for 1 hour and 1% with exposure to the treatment for 4 hours. The increase in the values of this indicator creates the possibility to consider that the treated seedlings will have a better capacity to absorb water and nutrients from the soil. This can contribute to a more vigorous and healthy growth of the plants. Longer and better developed roots allow a more stable anchorage in the soil and a greater resistance to stress conditions, such as drought or soil conditions.

Research conducted by Botnarenco et al. (2011) on the germination of seeds of approximately 70 taxa of medicinal plants has shown that storing seeds for a period of 4-6 years has a negative impact on the germination quality. In most species, an extension of the germination duration from 6 to 12 days was found. However, in the case of some medicinal plant species studied (*Althaea officinalis* L., *Amaranthus cruentus* L., *Carthamus tinctorius* L., *Coriandrum sativum* L., *Cephalophora aromatica* Gr., *Cynara scolymus* L., *Hyssopus officinalis* L., *Potentilla erecta* L.), storing seeds for 4-6 years did not significantly influence the germination capacity (Botnarenco et al., 2011). Our results showed that storing seeds of *Phacelia tanacetifolia* Benth. (honeybee) for 4 years caused a quantitative decrease in the number of germinated seeds and, implicitly, in the germination quality.

The biostimulatory effects on seed germination and plant health improvement resulting from the use of wastewater or cyanobacterial biomass have been demonstrated in numerous specialized studies.

The application of cyanobacterial filtrates from *Nostoc calcicola* and *Anabaena flos-aquae* for seed treatment of wheat, soybean, and clover varieties led to an increase in seed germination rate of up to 97.49% compared to the control group, with seedlings exhibiting visibly improved health status. Clover seedlings recorded the highest increase in shoot length (100%) compared to all other treatments applied to the tested crops. Meanwhile, soybean seedlings treated with *Nostoc* filtrate showed a 96.67% increase in root length relative to the other experimental variants. Overall, the treatment of wheat, soybean, and clover seeds with cyanobacterial filtrates had a significant positive effect, enhancing germination and promoting healthy plant development across all analyzed crops (El-Shahat Wahdan, 2007).

Investigations conducted by Lakshmi and Annamalai (2008) have demonstrated that wastewater (filtrates) obtained from the cultivation of the cyanobacteria *Anabaena ambigua* Rao and *Oscillatoria foreaui* exerts a pronounced biostimulatory effect on the plant *Withania somnifera* Dunal.

This effect was evidenced by enhanced plant growth on clay-sandy soils, as indicated by increases in the length and diameter of lateral roots, as well as total biomass. These results are attributed to the assimilation of biologically

active compounds present in the wastewater derived from cyanobacterial cultivation. (Lakshmi & Annamalai, 2008).

The application of combined filtrates (wastewaters) obtained from the cultivation of the cyanobacteria *Anabaena variabilis, Aulosira fertilissima, Nostoc muscorum*, and *Tolypothrix tenuis* on BG-11 nutrient medium had a significant impact on wheat seed germination. In the variant treated with wastewater, the germination rate reached 95%, compared to only 58.33% in the untreated control. Additionally, notable improvements were observed in germination speed and the seedling vigor index (Kumar & Kaur, 2014).

Research conducted by Zosim et al. (2021) has demonstrated that the filtrate obtained from the cultivation of the cyanobacterium *Spirulina platensis* contains compounds with biostimulatory effects on seed germination, such as amino acids (4.12-30.14 mg/ml), gibberellic acid (25.00-110.00 mg/ml), and indoleacetic acid (6.78-8.23 µg/l). The application of this filtrate, diluted with distilled water, to *Mentha spicata* seeds resulted in an increase in germination rate to 88%, compared to 68% in untreated seeds. A significant enhancement in seedling growth was also observed, with stem and root lengths being 2.12 and 2.15 times greater, respectively, than those of seedlings germinated without the addition of these biostimulants (Zosim et al., 2021).

In our research, the variants treated with the cyanobacterial biostimulant obtained by recycling wastewater from the cultivation of *Spirulina platensis* exhibited a quantitative increase in the number of germinated seeds, as well as a relative elongation of plant roots.

These results confirm the potential of cyanobacterial filtrates as an effective source of bioactive compounds with stimulatory effects on seed germination and early plant development.

CONCLUSIONS

The treatment of *Phacelia tanacetifolia* Benth. (Melifera) seeds with a biostimulator obtained from the wastewater resulting from the cultivation of the cyanobacterium *Spirulina platensis* has a positive effect on the germination capacity, especially in the variants of 1-2% biostimulator with exposure to the treatment for 1 hour. Older seeds (stored for 4 years) showed a reduction in the germination capacity, but the application of the biostimulator significantly improved the germination rate compared to the control variant. The germination index values confirm the stimulatory effect of the culture liquid, being significantly higher in the treated seeds compared to the untreated ones. The increase in the relative elongation of the roots following the treatment suggests an improved development of the root system, which may contribute to a more vigorous growth of the plants and a better adaptation to difficult environmental conditions.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

SURVIVAL RATE OF OAK (*Quercus* L.) SEEDLINGS IN AN AGROFORESTRY SYSTEM ESTABLISH IN HORTINOVA NURSERY (CÂRCEA, DOLJ COUNTY)

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Abstract

Agroforestry systems play a crucial role in mitigating the impacts of climate change, particularly in vulnerable regions like southeastern Romania, where drought poses a significant challenge to young forest stands. In 2023, twenty 24 m x 24 m plots were established, incorporating a mix of forest species and agricultural crops. Of these, 9 plots included four oak species, namely pedunculate oak (Quercus robur L.), red oak (Q. rubra L.), Turkey oak (Q. cerris L.), and sessile oak [Q. petraea (Matt.) Liebl.], as key tree species in an agroforestry experiment located in Cârcea, near Craiova, Dolj County. This study aimed to evaluate the survival rate of the planted oak seedlings. Additionally, climatic data (temperature, relative humidity and precipitation) were gathered using six HoBo sensors (Onset Computer Corporation) and one iMETOS 3.3 data logger. Soil analyses were done at Dolj Office of Pedological and Agrochemical Studies. The findings further highlight the significance of integrating oak species into agroforestry systems in southeastern Romania.

Key words*: agroforestry systems, Craiova, Dolj, oak, Quercus.*

INTRODUCTION

In the last decades, agroforestry systems have received increasing attention both from scientists and landowners. They are defined as complex mixed systems with a combination of crops, livestock and forest trees and shrubs (Paris et al., 2019).

By providing a broad variety of products for humans and animals (Mihăilă et al., 2021) and several ecosystem services, such as biodiversity preservation, soil erosion control, carbon sequestration, microclimatic moderator (Moreno et al., 2018; Lehmann et al., 2020; Santos et al., 2022; Budău et al., 2023; Dmuchowski et al., 2024), agroforestry systems are present worldwide.

Particularly, these systems could be established around the coastal rivers, to stabilize the banks, improve water quality and soil quality (Kachova & Dincă, 2015).

Recently, it was reported that agroforestry systems play an important role in tourism in India (Parthiban et al., 2023) and ecotourism in the wood pasture across Transylvanian Hills (Vijulie et al., 2024).

Shelterbelts are among the most common types of agroforestry systems, being composed of several rows of trees and shrubs (Mușat et al., 2024). Good practices were also reported in Bulgaria, where, for example, in a mixed poplar and agricultural crop area, it was reported that the soil characteristics improved significantly, especially in C and N content (Hinkov & Kachova, 2024). Moreover, by installing shelterbelts higher than 10 meters on 4-5% of the agricultural lands, the yield could increase by 30-50% (Mușat et al., 2021). Even so, only small areas are designated as agroforestry systems across Romania, two examples being the experiments recently done at RODAGRIA Ogru farm and National Agricultural Research and Development Institute Fundulea, where alley cropping were introduced with two rows of common hazel (*Corylus avellana* L.) and three rows of Siberian elm (*Ulmus pumila* L.) on an area of 38 hectares (Tudora et al., 2022).

In the perspective of climate change, oaks (Genus *Quercus* L.) are preferred in several combinations of agroforestry cultures, including agroforestry systems, such as in Central and Eastern Romania (Crișan et al., 2022), Spain (Pérez-Girón et al., 2022) and Slovakia (Stefancik & Pastor, 2023). Moreover, oak-based cultures/agroforestry systems are appreciated also in livestock grazing (Maruşca

et al., 2020; Wadud et al., 2024). In some cases, oak species could be a solution for afforestation of several categories of degraded lands, such as the sandy soils across southern-western part of Romania (Enescu, 2019; Enescu & Caradaică, 2023), but the selection of proper species should be done with caution taking into consideration that the Genus *Quercus* has a very complicated taxonomy, in some cases being very difficult to distinguish between closely related species (Curtu et al., 2011; Șofletea et al., 2011; Enescu et al., 2013). Oaks are very important also from the ecologic and economic perspective (Timiș-Gânsac et al., 2022), by providing a broad range of services and products, including non-wood forest products to humans (Abraham et al., 2015).

The aim of this study was to assess the survival rate of oak seedlings in an agroforestry system established in HortiNova Nursery.

MATERIALS AND METHODS

The experimental plot is situated in Cârcea, near Craiova (Dolj County; 44°16'53.6"N 23°55'37.7"E), within the HortiNova nursery, a private company.

The total area of 1.15 hectares was subdivided into 20 square plots, each featuring a unique combination of trees, shrubs, cereals, and vegetables. Among these, 9 plots were dedicated to oak-based tree and shrub combinations, with four oak species selected: pedunculate oak (*Quercus robur*), Turkey oak (*Q. cerris*), red oak (*Q. rubra*), and sessile oak (*Q. petraea*). The oak seedlings were grown at the HortiNova nursery during 2023 and were planted in November of the same year, in the same day, with the same workers and the same planting tools.

Oak seedlings were cultivated in containers (pots), with their roots contained within the potting medium (Figure 1).

Each subplot consisted of 11 or 12 rows, with 23 to 25 seedlings per row, spaced 2 meters apart between rows and 1 meter between seedlings within a row. On September 15, 2024, the seedlings were counted in each row across all 9 experimental subplots.

Climatic data was collected using six HoBo sensors and iMETOS 3.3 data logger. HoBo 1 was installed in plot no. 1, HoBo 2 in plot no. 3, HoBo 3 in plot no. 6, HoBo 4 in plot no. 9, HoBo 5 along the irrigation system, and HoBo 6 near plot no. 7 (Figure 2).

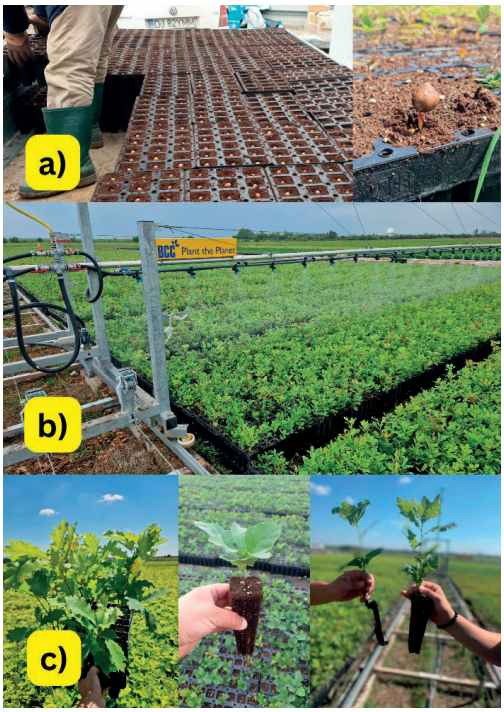


Figure 1. Producing oak seedlings with potted roots: a) placing the acorns into pots; b) regular and controlled watering of the potted seedlings; c) one-year-old oak seedlings in pots



Figure 2. Location of the 6 HoBo sensors
Source: Google Earth

In this survey, data on temperature (°C) and relative humidity (%) recorded by the HoBo 6

sensor, every 30 minutes, were compiled for the period from April 1st to September 15th, 2024, using Microsoft Office Excel.
Each HoBo sensor (model U23-001A Data Logger U23 Pro v2 Temperature/Relative Humidity) was placed at 1.5 meter above the soil level (Figure 3).



Figure 3. HoBo sensors

iMETOS 3.3 data logger was placed in the proximity of the experimental plots, 10 m away from plot no.3.
Soil analyses were conducted in plots no. 2 and 5 by the Dolj Office of Pedological and Agrochemical Studies, measuring pH (soil reaction), humus content (%), total nitrogen (%), as well as P and K (ppm).

RESULTS AND DISCUSSIONS

In total, 1.817 oak seedlings were planted (761 pedunculate oaks - "**P**", 227 sessile oaks - "**G**", 178 Turkey oaks - "**C**" and 651 red oaks - "**R**", respectively), out of which 1.486 survived after the first year (**81.7%**).
In 2024, the total recorded precipitation amounted to 238.8 mm (Figure 4).

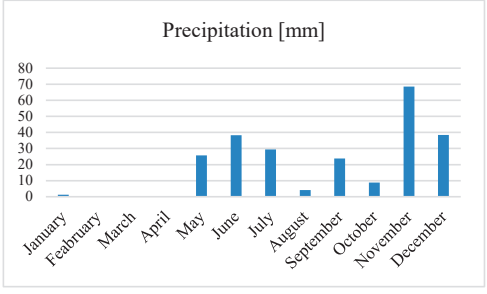


Figure 4. Monthly precipitation recorded in 2024

Nearly half of this rainfall occurred during the last three months of the year.
During the specified period, temperatures varied between 1.65°C and 39.13°C, with an average of 21.89 ± 7.23°C.
Regarding relative humidity (RH), values ranged from 17.95% to 88.53%, with an average of 54.50 ± 17.47% (Figure 5).

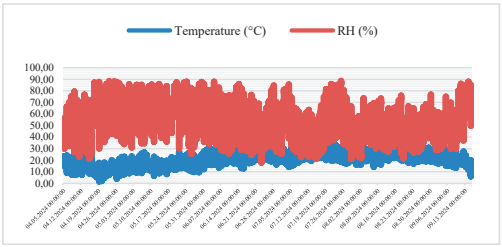


Figure 5. Temperature (°C) and relative humidity (%) recorded from 1st of April to 15th of September 2024

Soil analysis results are presented in Table 1.

Table 1. Results of soil analysis

Plot	pH	Humus (%)	N (%)	P (ppm)	K (ppm)
2	5.83	3.16	0.16	66	242
5	5.75	2.76	0.14	20.8	246

These results indicate poor soil quality, as the ideal pH range is between 6.0 and 7.5.
According to the Romanian Methodology for Soil Studies (Florea et al., 1987), the humus content is low, the nitrogen (N) content is moderate, and the phosphorus (P) and potassium (K) contents are moderate to high.
Figure 6 illustrates the results from plot no. 2, with the seedlings that did not survive highlighted in red. A total of 275 oak seedlings were planted, including 135 pedunculate oaks, 65 Turkey oaks, 38 sessile oaks, and 37 red oaks. Of these, 16 pedunculate oaks, 7 Turkey oaks, and 8 sessile oaks did not survive.

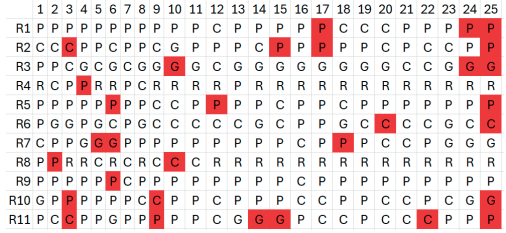


Figure 6. Distribution of oak seedlings in plot no. 2

Figure 7 displays the results from plot number 5, where 250 pedunculate oak seedlings and 50 Turkey oak seedlings were planted. Of these, 82 pedunculate oaks and 8 Turkey oaks did not survive.

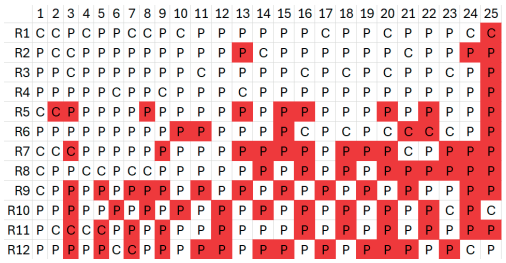


Figure 7. Distribution of oak seedlings in plot no. 5

In plot number 10, 136 red oak seedlings and 8 sessile oak seedlings were alternately planted alongside 144 Siberian elm (*Ulmus pumila* L.) seedlings, marked with "U". Of these, only 28 red oaks and 3 sessile oaks failed to survive (Figure 8).

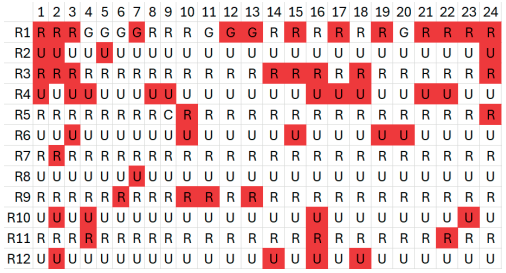


Figure 8. Distribution of oak seedlings in plot no. 10

In plot number 11, 138 pedunculate oak seedlings (marked "P"), 92 wild privet (*Ligustrum vulgare* L.) seedlings (marked "L"), and 23 Siberian elm (*Ulmus pumila* L.) seedlings (marked "U") were planted alternately. Of these, 23 pedunculate oaks, 34 wild privets, and 9 Siberian elms did not survive (Figure 9).

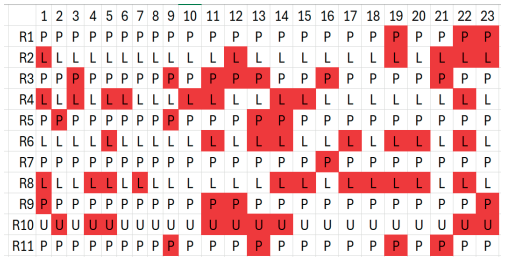


Figure 9. Distribution of oak seedlings in plot no. 11

Figure 10 displays the results from plot number 12, where 271 red oak seedlings, 3 Turkey oak seedlings and 2 pedunculate oak seedlings were planted. Of these, 51 red oaks did not survive.

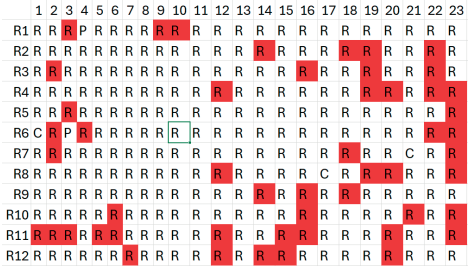


Figure 10. Distribution of oak seedlings in plot no. 12

In plot number 13, 156 sessile oak seedlings, 27 pedunculate oak seedlings, 81 honey locust (*Gleditsia triacanthos* L.) seedlings (marked "Gl") and 15 field maple (*Acer campestre* L.) seedlings (marked "J") were planted. Of these, only 2 sessile oaks and 48 honey locust seedlings failed to survive (Figure 11).

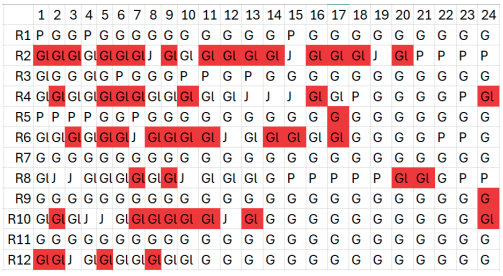


Figure 11. Distribution of oak seedlings in plot no. 13

Figure 12 displays the results from plot 14, where 171 red oak seedlings, 3 pedunculate oak seedlings, 16 sessile oak seedlings and 86 dogwood seedlings (marked "S") were planted. Of these, 20 red oaks, 2 sessile oaks and 31 dogwood seedlings did not survive.

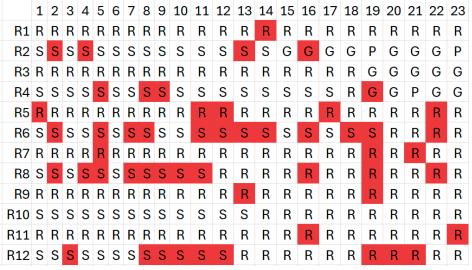


Figure 12. Distribution of oak seedlings in plot no. 14

In plot number 15, 9 sessile oak seedlings, 68 pedunculate oak seedlings, 60 Turkey oak seedlings, 36 red oak seedlings and 103 sycamore maple (*Acer pseudoplatanus* L.) seedlings (marked "Pa") were planted. Of these, only 2 sessile oaks, 8 pedunculate oaks, 7 Turkey oaks, 6 red oaks and 23 sycamore maples failed to survive (Figure 13).

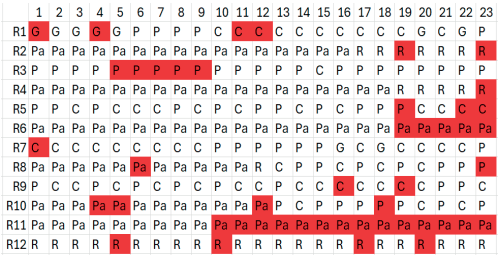


Figure 13. Distribution of oak seedlings in plot no. 15

In the last oak-based plot (*i.e.* plot no. 17), 138 pedunculate oak seedlings and 138 green ash (*Fraxinus pennsylvanica* Marshall) seedlings (marked "F"). Out of these, 58 pedunculate oaks and 18 green ashes did not survive (Figure 14).

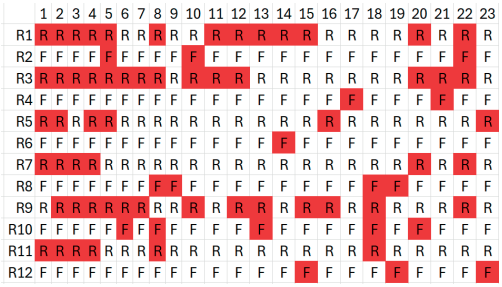


Figure 14. Distribution of oak seedlings in plot no. 17

Out of the 761 pedunculate oak seedlings planted, 187 (24.6%) failed to survive. The survival rates for the other oak species were higher, with red oak having a mortality rate of 16.1%, Turkey oak 12.4%, and sessile oak 7.5%.

CONCLUSIONS

Despite the challenging site conditions, including poor soil quality and rainfall, the survival rates for all four oak species were quite high. In 2024, nearly half of the total precipitation occurred in the last three months of the year, suggesting that fluctuating rainfall patterns likely influenced seedling survival, especially during periods of drought or excessive moisture.

The performance of **sessile oak** was particularly impressive, as this species typically prefers conditions with higher precipitation. These results indicate that sessile oak is the most resilient species under the given conditions. However, overall survival could be further improved with better soil quality and a more strategic planting approach that reduces competition and ensures adequate water and nutrients.

Although the production costs for potted root seedlings are higher than those for traditional bare-root methods, the outcomes of this study are promising. With only an 18.3% loss, this suggests that replanting needs will be lower, potentially leading to a significant reduction in future costs.

ACKNOWLEDGEMENTS

The research was financed by Tomorrow's Forests Foundation (padureademaine.ro). The author is also grateful to Marian Mechenici and Corina Ciaușu (HortiNova nursery) for their support.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

TRACKING THE PERCENTAGE OF SEED GERMINATION WHEN THE BASIS FOR THE SPROUTS IS PURE COMPOST AND COMPOST WITH ADDITIVES

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Abstract

The present study aimed to monitor the germination percentage of radishes and spinach in compost mass in pure form and with additives. Six variants and one control (V0) were prepared: V1 - lavender extract, V2 - thyme extract, V3 - basil extract, V4 - mix, V5 - microbial fertilizer, V6 - mineral fertilizer, V7 - Min. + microbial fertilizer for each of the studied crops - a total of 14 samples. An initial reading of the microbial community was carried out after the introduction of the additives and immediately before planting the seeds and then at day 5, day 10 and day 15. The temperature, humidity and pH of the samples were measured. The two considered crops give different germination percentages of the different variants. Spinach seeds generally had difficulty germinating and produced fewer sprouts than radishes. The best results for germination and survival of the sprouts were obtained for the spinach in the control and in the variant with mineral fertilizer and the variant with basil. For the radishes, the best germination was shown by the control and by the variants with microbial fertilizer, the variant with microbial and mineral fertilizer.

Key words*: microorganisms, compost, germination.*

INTRODUCTION

Seed germination and seedling preparation are key moments in agriculture. Seed germination is an important process that is influenced not only by external factors, but also by the physicochemical properties of the seed covering tissues (Martin et al., 2010). In their study, Baskin & Baskint et al. (2014) point out that the main factors influencing germination are temperature, water content, the presence of good aeration, light, etc. Some studies have focused on the limiting effect of some environmental factors on seed germination. Thus, in their study, Ghosh et al. (2014) indicated high salinity as detrimental to the development and germination of radish. Temperature is another important factor that can stimulate or inhibit seed germination (Abdel et al., 2016). In a number of studies, temperature and humidity have been identified as the main limiting factors, given their direct involvement in the activation and inactivation of various enzymes responsible for initiating the germination process (Bradford, 2002; Khaeim et al., 2022). In practice, a number of methods for pre-treatment of seeds are applied, with the aim of their easier and more sustainable germination (Kanjevac et al., 2022). It has also been found that the application of bio-priming with certain strains of bacteria increases the germination of radishes (Kaymak et al., 2009). Unfortunately, sometimes germination is unsuccessful due to the use of an unsuitable substrate, often contaminated with certain pathogens that can cause damping off of the seeds. There are a number of articles that emphasize the need for a sterile environment for germination precisely to avoid the unwanted weakening, and sometimes death, of already germinated seeds as a result of pathogenic species (Davoudpour et al., 2020). However, the procedures are often crop-specific and may result in damage to the treated seeds themselves (Sauer & Burroughs, 1986). In general, sterile media ensure the purity of germinating seeds, including the possible presence of pathogens. However, the provision of sterile media is associated with certain economic costs. The use of compost mixes is a potential solution. The positive changes that occur when compost is applied have been established in a number of studies (Hassan et al., 2023). However, there are certain aspects in which compost fertilization, for example, leads to a lower number of inflorescences in lavender compared to plants fertilized with mineral fertilizer (Manescu &

Dudau). Compost has a wide range of applications. However, pathogens are sometimes found in the finished compost, despite the high temperature levels during the thermophilic phase (Gurtler et al., 2018). Given the above, it is advisable to use a compost mix without the presence of animal manure, which minimizes the risk of potential contamination with pathogens.

Another problem mentioned in the literature is the structure and density of the compost, which hinders germination due to the resistance exerted by the roots. It is important to note that similar problems can occur when using poor quality compost and one that has not reached maturity. Quality and mature compost has a soil-like structure of the aggregates. The possibility of using compost as a germination base is related to the final characteristics of the compost. Thus, in their study (Yang et al., 2021), point out the potential toxicity of compost with a predominant share of cow manure, towards germination. Such toxicity is almost completely absent in mature compost, the starting substrates of which are only plant residues. However, the potential moment for the presence of certain pathogens in compost produced on the territory of agricultural farms remains.

There are previous studies of ours that address the possibility of compost decontamination by adding certain plants or plant aqueous extracts, such as lavender, thyme, etc. (Grigorova-Pesheva et al., 2024). Similarly, in their study, Barthod et al., 2018, indicate the possibility of using specific additives that can suppress the development of pathogenic species and improve the quality of the finished compost. Given that compost is an easily generated product from waste plant residues, its use as a basis for seed germination is a prerequisite for reducing costs for any farm. For the present study, radishes and spinach were chosen considering that the first crop is fast-germinating, and the second is indicative of the condition and characteristics of the soil.

The main goal of this study was to track the germination rate of seeds using pure compost and compost with additives as seed base.

MATERIALS AND METHODS

For the purpose of this study, spinach and radish seeds were used. Compost material was placed in sterile plastic containers to serve as a base for seed germination. The parameters of the ready-made compost used for seed germination were previously determined as follows: C:N ratio was 14:1, the acidity of the medium was neutral to slightly alkaline - 7.2, the content of total nitrogen and organic carbon was 1.76% and 4.64%, respectively, the sieving of the compost showed that it had a suitable structure и. The initial microbiological characteristics of the starting compost were determined, including the total microbial number and the amount of individual microbiological groups. Then the compost was distributed among the experimental vessels and the additives were introduced. For the purpose of the experiment, six variants and one control were prepared for each of the tested cultures. The variants are as follows: V0 - control, V1 - lavender extract, V2 - thyme extract, V3 - basil extract, V4 - mix, V5 - microbial fertilizer, V6 - mineral fertilizer, V7 - Min. + microbial fertilizer. A total of 14 containers were prepared, six variants for spinach and six identical variants for radishes + two controls. The temperature and humidity were measured daily in order to maintain optimal moisture levels necessary for the germination process. The acidity of the medium was recorded. In the first six variants, ten high-quality radish seeds were placed, and in the second, ten high-quality spinach seeds. For each of the variants, the total microbial number and the quantity of individual microbiological groups were recorded on the 5th, 10th and 15th day in separate identical containers in order not to damage the seeds. To determine the amount of non-spore-forming bacteria, nutrient agar was used, and the cultivation was carried out at 24°C for 2 days. To determine the bacilli, the samples were pre-pasteurized, with subsequent inoculation on nutrient agar and cultivation under the same conditions. To determine the fungi and actinomycetes, Čapek dox agar and Starch ammonia agar were used, respectively. Cultivation was for 7 days at the corresponding temperatures - 35°C for actinomycetes and 30°C for fungi. The results were averaged based on five-fold measurements. The standard deviation was statistically distributed. The number of germinated seeds for each variant was determined, as well as their condition.

RESULTS AND DISCUSSIONS

The results of microbiological analyses are presented in Table 1. In all variants, including the control, we have an increase in microbial abundance. Compared to the control, the increased amount of microorganisms is due to the introduction of water, for the purpose of comparability with the other results, in which optimal humidity is maintained.

Table 1. Main microbiological parameters (CFU*10^6/g compost)

	Variants	TMN	Non-spore-forming bacteria	Spore-forming bacteria	Actinomycetes	Mucoromycetes	Temperature (°C)	pH	Relative humidity (%)
Initial phase		3.93	2.15	0.60	0.27	0.30	21.2	6.56	30.80
5th day	C	6.05±0.83	3.18±0.89	1.01±0.11	0.81±0.30	0.05±0.06	21.5	6.47	31.7
	V1	6.47±0.83	3.28±0.89	1.11±0.11	0.87±0.30	0.10±0.06	21.0	6.53	30.8
	V2	6.65±0.83	3.19±0.89	1.29±0.11	0.77±0.30	0.10±0.06	21.1	6.48	32.6
	V3	6.69±0.83	3.19±0.89	1.09±0.11	1.18±0.30	0.13±0.06	21.0	6.31	31.6
	V4	6.13±0.83	3.18±0.89	0.91±0.11	1.01±0.30	0.13±0.06	21.1	6.39	31.7
	V5	5.54±0.83	1.44±0.89	1.09±0.11	1.68±0.30	0.24±0.06	21.5	6.57	30.4
	V6	4.37±0.83	1.38±0.89	1.01±0.11	0.87±0.30	0.10±0.06	22.0	6.52	31.0
	V7	4.99±0.83	1.66±0.89	1.09±0.11	1.06±0.30	0.08±0.06	22.0	6.47	29.3
10h day	C	5.91±0.53	2.52±0.43	1.08±0.14	1.18±0.25	0.07±0.02	22.5	6.54	48.25
	V1	6.45±0.53	3.29±0.43	1.11±0.14	0.87±0.25	0.07±0.02	22.0	6.50	48.74
	V2	7.14±0.53	3.33±0.43	1.43±0.14	0.84±0.25	0.12±0.02	22.4	6.54	49.20
	V3	6.05±0.53	3.28±0.43	1.06±0.14	0.59±0.25	0.07±0.02	22.0	6.48	48.02
	V4	6.40±0.53	3.24±0.43	0.92±0.14	1.21±0.25	0.10±0.02	22.0	6.30	48.75
	V5	5.63±0.53	2.18±0.43	1.09±0.14	1.18±0.25	0.08±0.02	23.0	6.42	49.55
	V6	6.12±0.53	3.02±0.43	1.06±0.14	0.87±0.25	0.10±0.02	22.5	6.50	52.01
	V7	7.07±0.53	3.31±0.43	1.14±0.14	1.34±0.25	0.13±0.02	22.5	6.47	50.17
15h day	C	6.00±0.59	2.55±0.43	1.09±0.14	1.18±0.23	0.08±0.03	22.0	6.54	50.25
	V1	6.84±0.59	3.38±0.43	1.18±0.14	1.01±0.23	0.10±0.03	22.7	6.49	51.74
	V2	7.39±0.59	3.36±0.43	1.46±0.14	0.97±0.23	0.13±0.03	22.5	6.48	50.24
	V3	6.72±0.59	3.36±0.43	1.16±0.14	0.94±0.23	0.10±0.03	22.6	6.51	50.35
	V4	6.03±0.59	3.29±0.43	0.97±0.14	0.67±0.23	0.12±0.03	22.4	6.30	51.00
	V5	5.95±0.59	2.27±0.43	1.16±0.14	1.24±0.23	0.12±0.03	22.5	6.42	49.89
	V6	6.50±0.59	3.16±0.43	1.13±0.14	0.97±0.23	0.12±0.03	22.5	7.20	51.11
	V7	7.41±0.59	3.39±0.43	1.21±0.14	1.43±0.23	0.17±0.03	22.8	6.52	50.36

Optimal humidity is the only controlled parameter, in order to ensure optimal conditions for seed germination. The microbial number data show interesting dynamics (Figure 1). On the fifth day of the experiment, all of the studied variants had a higher microbial number compared to the initial phase. Variant V2 with thyme and V3 with basil stood out with the greatest increase in microbial abundance. The microbial number of the variant with introduced mineral fertilizer increased the least. Similar data indicate certain difficulties for the autochthonous microflora when introducing mineral fertilizer into the compost. This is probably due to the moment of adaptation and the specificity of its absorption by the microbial community.

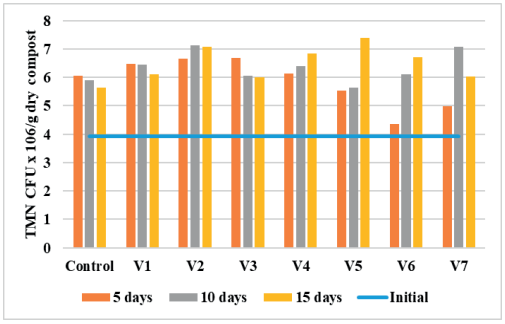


Figure 1. Total microbial number of the tested variants

The variant with only a microbial supplement, as well as the variant with a mix of mineral and microbial supplements, also provide a smaller increase in the total number of microorganisms compared to the variants with plant-based aqueous extracts.

Seed analysis showed initiation of germination in radish seeds, while no development was observed in spinach seeds by day 5. This is an expected result, given that spinach seeds require a longer germination phase.

On the tenth day, the amount of microorganisms in the control decreases. For the variants with plant extracts, we have different dynamics. For Variant 1 and Variant 3, we observe a very slight decrease in microbial abundance, while for Variants 2, 4 and 5, an increase in microbial abundance is observed. The variant with the microbial supplement also shows a slight increase. The data for Variant 6 are interesting, which on the fifth day had the lowest recorded microbial number. On the tenth day, a visible increase in microbial abundance is observed. This indicates that the microbial biota has adapted and has begun to absorb and transform the supplement introduced into the environment. Variant 7 with a mix of microbial and mineral supplement shows the greatest increase in the total microbial number. Variant 2 and Variant 7 stand out with the highest microbial abundance on the tenth day.

On the 10th day after sowing, a higher number of well-developed radish sprouts were observed in the Control (100% germination), Variant 5 (100% germination) and Variant 7 (100% germination). In spinach, sprouts began to develop in the Control (60% germination), Variant 3 with basil (40% germination) and Variant 6 with mineral fertilizer (40% germination). In general, radishes germinate more easily than spinach, regardless of the different speed. In radishes, nice, healthy and fresh sprouts are observed.

On day 15, we again have specific dynamics in the change of microbial communities in the different variants. In the control, the microbial abundance continues to decrease. In addition to the control, a decrease in total microbial number was also recorded in Variant 1, with lavender extract. The variant with a mix of mineral and microbial fertilizer also shows reduced levels of microbial abundance. With a higher total microbial number compared to day ten, V3, V4, V5 and V6 were recorded. Variant with microbial supplement showing the greatest increase.

On the fifteenth day, the trend in the development of sprouts is maintained. Spinach is experiencing visible difficulties. Some of the sprouts that have appeared show signs of damping off. There is a very low percentage of germination overall. Again, the control gives better results than the variants with additives (60% germination). This shows that the use of compost containing the studied additives is not appropriate for use as a basis for the germination of spinach sprouts. We believe that the pure compost used is also not suitable, since even in it the germination is not high enough. In addition, in all variants in which there are germinated seeds, including in the control, some of the sprouts show signs of damping off.

In radishes, there is a better germination rate, with only the variant with mineral fertilizer showing certain signs of damping off. This is also the variant with the lowest germination rate (40%). The variant with lavender (50%) also had a relatively low germination rate. On day 15, the variants with the highest germination rate for radishes were the Control (100% germination, with well-formed and fresh sprouts), the Variant with microbial supplement (100% germination, with well-formed and fresh sprouts), the Variant with mineral and microbial fertilizer (100% germination, with well-formed and fresh sprouts). Variant 3 with basil (90%) and Variant 2 with thyme (80%) showed very good germination. The generated data show that, unlike spinach, compost in its pure form, as well as with additions of mineral and microbial fertilizer, as well as with only a microbial additive, are a good basis for radish germination.

The present study did not find a direct correlation between total microbial number and germination percentage.

For a more detailed analysis, the change in the percentage of individual microbiological groups in the experimental variants was monitored (Figure 2). In the initial phase, the group of non-spore-forming bacteria is dominant. The data show that on day 5, in all variants including the control, the percentage of micromycetes decreases dramatically. This is probably due to the reduced aeration in the containers. Micromycetes are highly sensitive to reduced aeration, in addition, the increased humidity, in order to germinate the seeds, probably also had an impact on this microbial group. Of interest are the data from Variants 5, 6 and 7. In them, after the introduction of the additive, we have a decrease in the percentage of the group of non-spore-forming bacteria at the expense of the development of actinomycetes and bacilli.

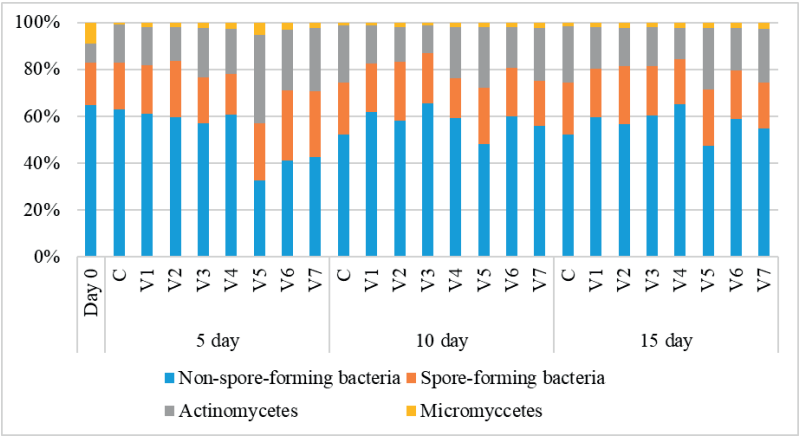


Figure 2. Percentage participation of individual microbial groups

This is most strongly expressed in Variant 5 with microbial supplement. Such a sharp change in the percentage of the main microbiological groups is associated with the introduction of microflora that is allochthonous to the compost mass, which temporarily enters into competitive relations with allochthonous microorganisms. Given that bacteria make up a major percentage of the microbial community, they experience this influence the most. Probably because of this, actinomycetes have temporarily increased their percentage. On the tenth and 15th day, a uniformity of the microbial community is observed, regardless of the studied variant. Again, the group of non-sporeforming bacteria dominates, followed by the group of bacilli and actinomycetes. Micromycetes occupy a minimal share in the microbial community. After performing an analysis for a potential relationship between the distribution of microbial groups and the degree of germination, no such relationship was found.

CONCLUSIONS

The present study aimed to track the percentage of seed germination when the basis for the sprouts is pure compost and compost with additives. The present study did not find a direct correlation between the total microbial number and the percentage of individual microbial groups and the germination percentage. During the experiment, the amount of total microflora in the control decreased over time.

The results for the experimental variants are heterogeneous. Thus, on the fifth day, the variants with imported plant water extracts showed a higher total microbial number compared to the control, while the variants with mineral and microbial additives showed lower values compared to the control. On the fifth day, we found signs of germination only in the variants with radishes.

On the tenth day, only Variants 1 and Variant 3 showed a minimal decrease in their microbial abundance, while an increase was recorded in

the others. On the 10th day after sowing, a higher number of well-developed sprouts were present in the Control (100% germination) and Variant 5 (100% germination) and Variant 7 (100% germination) for radishes.

In spinach, sprouts began to develop in the Control (60% germination), Variant 3 with basil (40% germination) and Variant 6 with mineral fertilizer (40% germination). In general, radishes germinate more easily than spinach, regardless of the different speed. Nice, healthy and fresh sprouts are observed in radishes.

On the 15th day, microbial abundance continues to decrease in the control and Variants 1, 3 and 7.

On the fifteenth day, the data show the appearance of damping off in a large part of the spinach sprouts. Again, the control gives better results than the variants with additives (60% germination). This shows that the use of compost containing the studied additives is not appropriate for use as a basis for the germination of spinach sprouts.

In radishes, there is a better germination rate, with only the variant with mineral fertilizer showing certain signs of damping off. This is also the variant with the lowest germination rate (40%). On day 15, the variants with the highest germination rate for radishes are the Control, the Variant with microbial additive and the Variant with mineral and microbial fertilizer.

The generated data show that, unlike spinach, compost in its pure form, as well as with additives of mineral and microbial fertilizer, as well as with only microbial additive, are a good basis for the germination of radishes.

In conclusion, we believe that compost in its pure form, as well as with imported mineral and microbial additive can be used for the germination of radishes, but not spinach. Overall, further studies are needed on a wider range of crops to enrich the knowledge on the possibility of using compost and compost with additives as a germination medium.

The present study can be used as a basis for future similar studies, as well as a basis for various developments related to the influence of different additives on the microbial community of finished compost.

ACKNOWLEDGEMENTS

The current study was performed in the framework of 'Investigating interrelationships in different variations of organic waste composting, by creating dynamic compost mixtures with the addition of ameliorants and tracking microbiological and enzyme activity - variants for applying a quality organic-mineral compost-based improver in agricultural practice' – КП-06-Н66/10" funded by Research Fund of Ministry of Education and Science in Bulgaria.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

HARMFUL AND BENEFICIAL FAUNA ASSOCIATED WITH MEDICINAL AND AROMATIC PLANTS

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Abstract

Our country has a wide variety of wild medicinal and aromatic plants and very good conditions for their cultivation. Due to the diversity of active principles they contain, they represent an impressive source of plant material for medicinal, food and other uses, being a significant natural resource and an important part of our cultural and scientific heritage. Most medicinal and aromatic plants are entomophilous, their reproduction depending on pollinating insects. This paper presents the harmful and beneficial species associated with medicinal and aromatic plants, present in the didactic collection of USAMV Bucharest, in the period 2023-2024. Species belonging to the classes Arachnida, Insecta, Gastropoda, Amphibia and Reptilia were identified. Species such as Rhopalapion longirostre Olivier, Nezara viridula L., Chrysolina coerulans Scriba, Agapanthia cynarae Germar, Pegomya terebrans Rondani and Aphis spp. showed a significant attack on host plants. Beneficial fauna was also identified and evaluated.

Key words*: medicinal plants, aromatic plants, host plants, harmful fauna, beneficial fauna.*

INTRODUCTION

Due to their chemical composition, medicinal and aromatic plants have a wide use for pharmaceutical and food purposes (Luchian et al., 2017). Organic farming systems recommend them as companion plants in various horticultural crops, as a source of pollinators and beneficial fauna (Roman et al., 2009). It is recommended that plants with male-sterile, dioecious or partially self-fertile flowers, with entomophilous pollination, be associated with medicinal and aromatic plants, in various crop systems, to increase the percentage of fruit setting (Burns & Stanley, 2022). Medicinal and aromatic plants also easily find a place in private ornamental gardens, patios, gardens on the roofs, balconies, in front of kitchen windows, or on sloped areas (Arslan & Yanmaz, 2010), being often marketed by specialized units, alongside ornamental plants. There is growing interest in potted aromatic plants for culinary and decorative purposes (Krisantini et al., 2022).
Some species are the basis for obtaining pesticides of plant origin, with a role in preventing the establishment, development and control of harmful organisms (Niroumand et al., 2016). Data on the harmful fauna associated with medicinal and aromatic plants are briefly presented in the specialized literature in Romania (Păun et al., 1986). However, these plants, in addition to the abundant beneficial fauna, are also populated by harmful species, which can depreciate their quality (Mazunder & Anouke, 2023; Rău et al., 2024). Knowledge and control of harmful fauna is essential for obtaining plants rich in active principles with a therapeutic role (Mitituc et al., 2000).

MATERIALS AND METHODS

The observations were carried out in the collection of medicinal and aromatic plants of USAMV of Bucharest, Romania, during 2023-2024. It includes over 40 species of perennial and annual plants (Figure 1).
The vast majority of the species are native plants, with high potential for large-scale cultivation, adapted to the climatic conditions of our country.



Figure 1. Planting scheme in the collection of medicinal and aromatic plants of USAMV of Bucharest

Our observations aimed at qualitative assessment of harmful and beneficial fauna. Species were detected by weekly visual inspection; with the help of yellow adhesive traps for flaying species, pheromone traps and by installing Barber traps.

The atraSEG pheromone trap was used to detect the *Agrotis segetum* Denis & Schiff species. Barber traps were installed at ground level, remaining open 24 hours/month, so as not to destroy the beneficial fauna.

Harmful and beneficial entomofauna was collected and determined in the laboratory, based on specific morphological characters at the genus and species level.

The results are expressed in the form of lists of harmful and beneficial species detected on medicinal and aromatic plant species.

RESULTS AND DISCUSSIONS

Following observations made in the collection of medicinal and aromatic plants of USAMV of Bucharest, during the period 2023-2024, 25 harmful species belonging to the classes Arachnida, Insecta and Gastropoda were detected. Regarding the structure of the detected species, the highest share was recorded by the class Insecta (88%), followed by the class Gastropoda (8%) and Arachnida (4%). Within the class Insecta, the species of the order Hemiptera recorded 36.36% of the total insect species, followed by the order Lepidoptera with 27.27%, and Coleoptera with 22.72%. The orders Orthoptera, Thysanoptera and Diptera each represented a share of 4.54% of the total species detected (Figure 2).

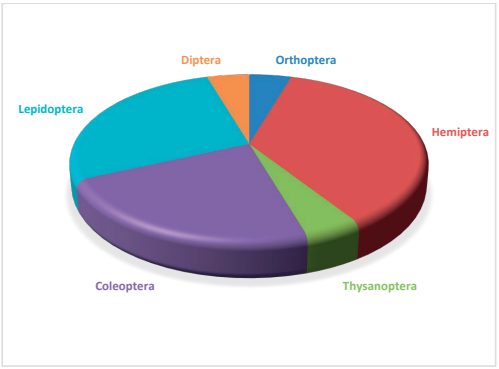


Figure 2. The structure of insect pests

I. All the pest species detected are present in Europe and in our country (Table 1). Most of them are known as polyphagous pests (*Tetranychus urticae* Koch., *Gryllotalpa gryllotalpa* L., *Psammotettix alienus* Dahlb., *Corizus hyoscyami* L., *Thrips tabaci* L., *Oxythyrea funesta* Poda., *Agrotis segetum* Denis & Schiff., *Iphiclides podalirius* L., *Helix pomatia* L., *Deroceras agreste* L.). Rău et al. (2024) present 8 polyphagous species, of which 3 (*Tetranychus urticae* Koch on plants of *Echinacea purpurea* Moench, *Thrips tabaci* L. on plants of *Rosa* spp. and *Nezara viridula* L. on plants of *Althaea rosea* L.) are also found in our observations. Other species are narrowly polyphagous (*Phyllotreta atra* Fabricius on plants of *Mellisa officinalis* L.) or are oligophagous species (*Graphosoma lineatum* L., on plants of *Feniculum vulgare* Mill., *Pegomya terebrans* Rondani on plants of *Cynara scolymus* L.).

Aphididae species were found on mint, sage and in high density on artichoke plants, stationed on the growth tips and inflorescences (Figure 3, a-d). Aphid colonies were accompanied by Coccinellidae and *Formica* spp.

Two species, *Nezara viridula* L. and *Halyomorpha halys* L., are found on the list of invasive species and with a pronounced polyphagism (CABI Compendium Invasive Species). *Nezara viridula* L. known in Romania since 2012 (Grozea et al., 2012; 2015) was present on sage and mallow *Althaea rosea* L. plants (Figure 3 h; 3 i), being reported in the literature on 6 species of Malvaceae (CABI Compendium; Gutue et al., 2024). *Halyomorpha halys* L. present in Romania since 2015 (Macavei et al., 2015) was detected on buckthorn plants *Hippophae rhamnoides* L., the host plant for reproduction and food (Zakharchenko et al., 2020; CABI Compendium).

The weevil *Rhopalapion longirostre* Olivier, an oligophagous species (Plant parasites of Europe) recorded high densities on *Althaea rosea* L. plants. This weevil has been present in our country since 1875 and was reported in Oltenia on *Alcea rosea* L. plants, synonymous with *Althaea rosea* L. (Bârcă et al., 2011).

Agapanthia cynarae Germar is a xerothermal beetle species, present in South-Eastern Europe, polyphagous on herbaceous plants. In our country, it was reported in the Danube Gorge, in 1976 by Oreva and Slătinic (Serafim, 2010). Adults were present on artichoke plants at the beginning of June (Figure 3 l). The larvae developed at the base of the stem where they produced galleries (Figure 3 m), being detected in the fall when the crop was cleared, by sectioning the stems.

Lepidoptera species were present in the collection of medicinal and aromatic plants, as adults, feeding on flower nectar.

Chrysolina coerulans Scriba reported in Romania in 2010 (CABI compendium Invasive Species) and 2017-2019 (Ilie & Abrudan, 2019), produces gnawing on the growth tips of mint plants (Figure 3 p).

Pegomya terebrans Rondani larvae produced mines in the leaf blade of artichoke plants. At first, the mines are produced individually by each larva, then they merge and the presence of 2-3 larvae is observed. The upper epidermis becomes transparent and black excrement can be observed (Figure 3 x). It is an oligophagous species, reported in our country by Drăghia in 1968 (Plant parasites of Europe). Gastropods produced gnawing on the basal leaves of *Salvia sclarea* L., during periods of high humidity (Figure 3 y).

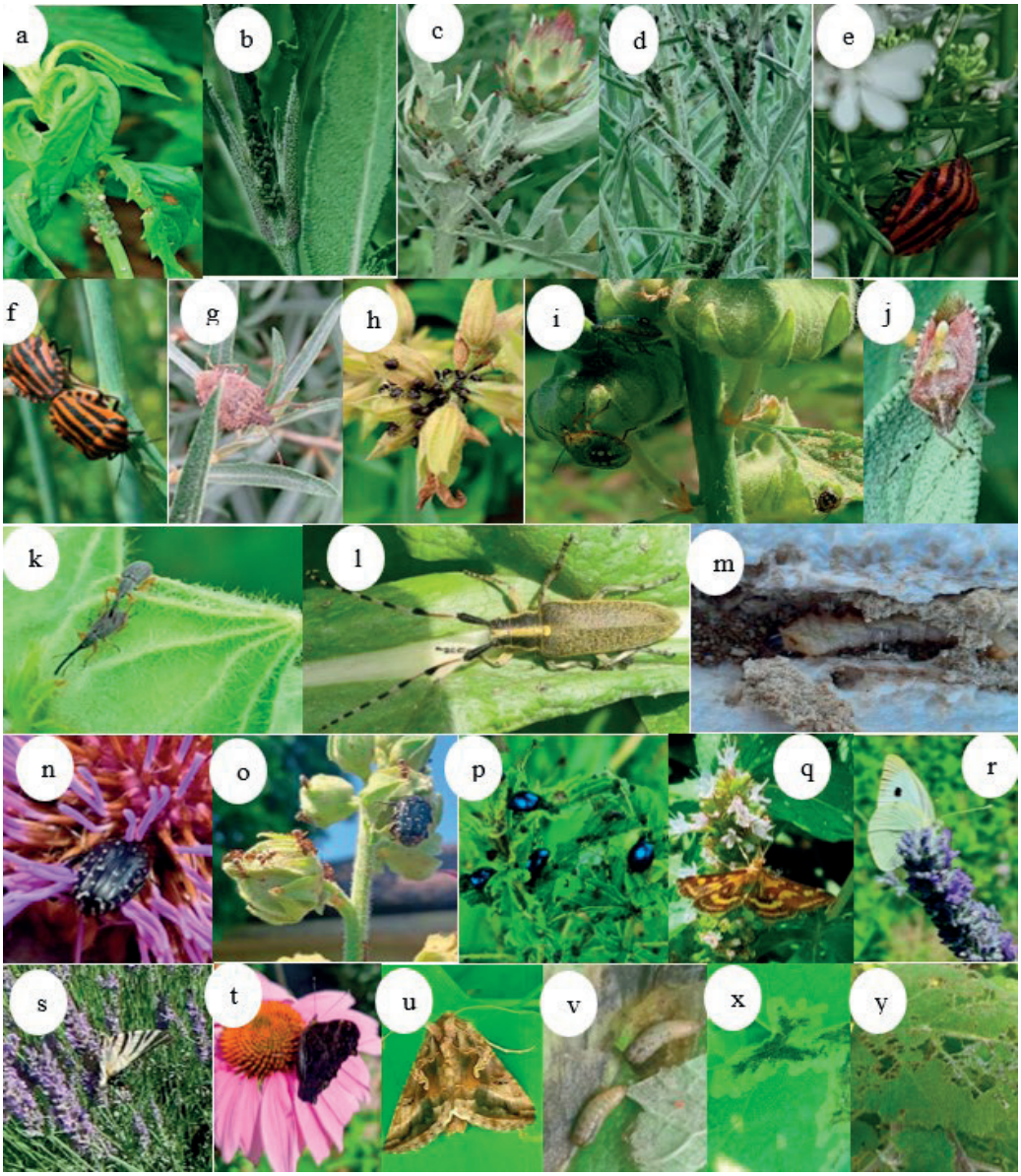


Figure 3. Harmful fauna associated with medicinal and aromatic plants: a, b, c, d - Aphididae on mint, sage and artichoke; e, f - *Graphosoma lineatum*; g - *Halyomorpha halys*; h, i - larvae of different stage of *Nezara viridula*; j - *Dolycoris baccarum*; k - *Rhopalapion longirostre;* l - *Agapanthia cynarae*; m - larva of *Agapanthia cynarae* in the stem of an artichoke plant; n, o - *Oxythyrea funesta* on flowers of different plants; p - *Chrysolina coerulans*; q - *Pyrausta purpuralis*; r - *Pieris brassicae*; s - *Iphiclides podalirius*; t - *Aglais io*; u - *Autographa gamma*; v - larvae of *Pegomya terebrans*; x - *Pegomya terebrans* mines; y - attack produced by Gastropoda

Table 1. Harmful fauna associated with medicinal and aromatic plants

Class	Order	Family	Species	Feeding regime of the pest/host plant
Arachnida	Acari	Tetranychidae	*Tetranychus urticae* Koch	polyphagous species
Insecta	Orthoptera	Gryllotalpidae	*Gryllotalpa gryllotalpa* L.	polyphagous species
	Hemiptera	Cicadellidae	*Psammotettix* alienus Dahlb.	polyphagous species
			Typhlociba rosae L.	*Rosa* spp. L.
		Aphididae	*Aphis* spp. L.	mint, sage, artichoke
		Scutelleridae	*Graphosoma lineatum* L.	umbelliferous
		Pentatomidae	*Nezara viridula* L.	polyphagous species
			Halyomorpha halys L.	polyphagous species
			Dolycoris baccarum L.	polyphagous species
		Rhopalidae	*Corizus hyoscyami* L.	polyphagous species
	Thysanoptera	Thripidae	*Thrips tabaci* L.	polyphagous species
	Coleoptera	Apionidae	*Rhopalapion longirostre* Olivier	oligophagous species
		Cetoniidae	*Oxythyrea funesta* Poda.	polyphagous species
		Chrysomelidae	*Chrysolina coerulans* Scriba	mint
			Phyllotreta atra Fabricius	narrowly polyphagous
		Cerambycidae	*Agapanthia cynarae* Germar	polyphagous in herbaceous plants/ artichoke
	Lepidoptera	Noctuidae	*Agrotis segetum* Denis & Schiff.	polyphagous species
			Autographa gamma L.	polyphagous species
		Crambidae	*Pyrausta purpuralis* L.	mint, sage, thyme, oregano
		Pieridae	*Pieris brassicae* L.	oligophagous species
		Papilionidae	*Iphiclides podalirius* L.	polyphagous species
		Nymphalidae	*Aglais io* L.	hop, nettle
	Diptera	Anthomyiidae	*Pegomya terebrans* Rondani	oligophagous species
Gastropoda	Stylommatophora	Helicidae	*Helix pomatia* L.	polyphagous species
		Agriolimacidae	*Deroceras agreste* L.	polyphagous species

II. Beneficial fauna was present on medicinal and aromatic plants from spring to early autumn. A variety of genera and species were observed and identified (Table 2).

The diversity of plants in the collection of medicinal and aromatic plants, as well as their different flowering periods, attracted a multitude of beneficial insect species throughout the observation period. In addition to the richness in nectar and pollen, even honeydew constitutes an important source of material for the attraction and development of beneficial pollinating fauna (Iordache et al., 2008). Pollinating species were frequently observed on plants such as *Lavandula angustifolia* Mill., *Lavandula x intermedia* L., *Echinacea purpurea* Moench. (L.), *Origanum majorana* L., *Salvia officinalis* L., *Cynara scolymus* L. and *Althaea rosea* L.

The detected insect species belong to the orders Hymenoptera, Diptera and Coleoptera. The vast majority are pollinating insects (Figure 4), but predatory zoophagous species were also present (Table 2).

Ladybird species (Figure 5) and Syrphidae larvae managed to keep aphid populations under control, with plants growing very well.

Table 2. Beneficial fauna associated with medicinal and aromatic plants

Class	Order	Family	Species	Feeding regime
Insecta	Hymenoptera	Apidae	*Apis mellifera* L.	Important pollinator of plants
			Bombus spp. Latreille	Important agricultural pollinators
		Scoliidae	*Scolia hirta* Schrenck	Feed on nectar and pollen Parazitoid species
		Formicidae	*Formica* spp. L.	Feed on honeydew
	Diptera	Bombyliidae	*Bombylius major* L.	Nectar robber Feed pollen Pollinator
		Syrphidae	*Syrphus* spp. Fabricius	Adults feed only on pollen, nectar, or honeydew Larvae are predatory, feeding on aphids
	Coleoptera	Coccinellidae	*Coccinella septempunctata* L.	Aphidophagous species, but also feeds on other arthropods, fungal spores, pollen
			Harmonia axyridis Pallas	Aphidophagous species, but also feeds on other arthropods, fungal spores, pollen, aphidophagous insects and fruits
			Adalia bipunctata L.	Aphidophagous species,but also feed other small insects, insects eggs and mites
		Scarabeidae	*Cetonia aurata* L.	Adults feed on pollen, nectar and flowers Larvae are saprophagous
		Dermestidae	*Anthrenus verbasci* L.	Adults feed on pollen and nectar Larvae feed on keratin and chitin of natural fibers
		Chrysomelidae	*Clytra laeviuscula* Ratezburg	Adults feed on pollen, nectar and leaves Larvae are found in ant nests
		Carabidae	*Carabus* spp. L.	Predatory species
			Pterostichus cupreus L.	Predatory species
			Pterostichus niger Sch.	Predatory species
Amphibia	Anura	Bufonidae	*Bufo bufo* L.	Feed on invertebrates
Reptilia	Squamata	Lacertidae	*Lacerta agilis* Laurenti	Feed on insects, spiders, earthworms and snails



Figure 4. Beneficial, pollinator species: a - *Bombylius major* L.; b - *Apis mellifera* L.



Figure 5. Beneficial, predatory zoophagous species (*Coccinellidae*)

CONCLUSIONS

In the collection of medicinal and aromatic plants of USAMV of Bucharest, 25 harmful species were detected: *Tetranychus urticae* Koch, *Gryllotalpa gryllotalpa* L., *Psammotettix* alienus Dahlb., *Typhlociba rosae* L., *Aphis* spp. L., *Graphosoma lineatum* L., *Nezara viridula* L., *Halyomorpha halys* L., *Dolycoris baccarum* L., *Corizus hyoscyami* L., *Thrips tabaci* L., *Rhopalapion longirostre* Olivier, *Oxythyrea funesta* Poda., *Chrysolina coerulans* Scriba, *Phyllotreta atra* Fabricius, *Agapanthia cynarae* Germar, *Agrotis segetum* Denis & Schiff., *Autographa gamma* L., *Pyrausta purpuralis* L., *Pieris brassicae* L., *Iphiclides podalirius* L., *Aglais io* L., *Pegomya terebrans* Rondani, *Helix pomatia* L. and *Deroceras agreste* L. The vast majority are polyphagous species, but oligophagous species have also been identified. Species such as *Rhopalapion longirostre* Olivier, *Agapanthia cynarae* Germar, *Chrysolina coerulans* Scriba, invasive stink bugs (*Nezara viridula* L., *Halyomorpha halys* L.) and aphids are pests that require monitoring and control management.

Beneficial fauna (pollinators and zoophagous) was represented by 17 species: *Apis mellifera* L, *Bombus* spp. Latreille, *Scolia hirta* Schrenck, *Formica* spp. L., *Bombylius major* L., *Syrphus* spp. Fabricius, *Coccinella septempunctata* L., *Harmonia axyridis* Pallas, *Adalia bipunctata* L., *Cetonia aurata* L., *Anthrenus verbasci* L., *Clytra laeviuscula* Ratezburg, *Carabus* spp. L., *Pterostichus cupreus* L., *Pterostichus niger* Sch., *Bufo bufo* L. and *Lacerta agilis* Laurenti.

The variety of beneficial species recommends medicinal and aromatic plants as species that favor their conservation.

Our observations contribute to obtaining more information about the harmful and beneficial fauna associated with medicinal and aromatic plants. They can form the basis for further

research in the field and can be useful for those involved in the cultivation and use of medicinal and aromatic plants in landscape design.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

BIOACTIVE POTENTIAL OF SPENT *Rosa damascena* Mill. PETALS EXTRACT: ANTIOXIDANT AND ANTIMICROBIAL PROPERTIES

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Abstract

The rose petal from Rosa damascena Mill. are often associated as a source of compounds with antioxidant activity and antimicrobial properties. The rose oil-bearing industry is generating enormous quantities of by-products with a potential to become part of the circular economy. Therefore, we aimed to produce an extract from the spent rose petals (SRPE) by-water: ethanol (70:30) extraction, followed by concentration and freeze drying. The phenolic profile evaluated by HPLC showed a presence of Gallic, Protocatechuic, p-Coumaric acids and Rutin. Also, the organic acid such as Ascorbic, Malic, Tartaric and Oxalic were found. The obtained extract exhibited a strong antioxidant activity evaluated by the radical scavenging activity against DPPH and ABTS radicals, metal chelating capacity against transition metal and oxygen and hydroxyl radical absorbance capacity (ORAC and HORAC). In addition to antioxidant activity, the extract manifested an antimicrobial activity against most of the pathogens responsible for food poisoning. The minimum inhibitory concentrations of rose extract for yoghurt and probiotic starter cultures were evaluated.

Key words*: by-product, rose extract, antioxidant activity, MIC yoghurt starter culture, MIC probiotic starter culture.*

INTRODUCTION

The growing consumer demand for natural bioactive compounds in food and pharmaceuticals leads to an increased research into plant-derived antioxidants and antimicrobials. *Rosa damascena* Mill., commonly known as the Damask rose, is widely cultivated for its essential oils and medicinal properties (Mahboubi, 2016). Beyond its traditional uses, *Rosa damascena* is a rich source of phenolic compounds, flavonoids, and organic acids, which have demonstrated significant antioxidant, anti-inflammatory, and antimicrobial activities (Dimitrova et al., 2017; Dinkova et al., 2022; Petkova et al., 2020).

Despite the high-value essential oil extracted from the petals, the rose oil industry generates large quantities of spent rose petals (SRP) as by-products, which remain largely underutilized (Boroski et al., 2021). Given the presence of bioactive compounds in SRP, there is a strong incentive to re-use these by-products in the circular economy by extracting their valuable secondary metabolites for potential food, pharmaceutical, and nutraceutical applications (Mohammadpour et al., 2023). Recent studies confirmed that *R. damascena* petals contain a variety of bioactive compounds, including phenolic acids (Gallic acid, Protocatechuic acid, p-Coumaric acid), flavonoids (Rutin, Quercetin), and organic acids (ascorbic acid, malic acid, tartaric acid, oxalic acid) (Charoimek et al., 2024; Dragoev et al., 2021). These compounds are known for their antioxidant properties, which are primarily attributed to their radical-scavenging capacity, metal ion chelation, and inhibition of lipid peroxidation (Bora et al., 2020).

The antioxidant activity of *R. damascena* extracts has been previously evaluated using DPPH (2.2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)), oxygen radical absorbance capacity (ORAC), and hydroxyl radical absorbance capacity (HORAC) assays, confirming its potential as a natural antioxidant in food preservation (Shang et al., 2019).

In addition to antioxidant activity, *R. damascena* extracts exhibit broad-spectrum antimicrobial properties against Gram-positive and Gram-negative bacteria, as well as fungi (Dimitrova & Ivanov, 2020; Mahmood et al., 2020). The antimicrobial activity is primarily attributed to

phenolic acids and flavonoids, which can disrupt microbial membranes, inhibit enzymatic activity, and interfere with bacterial quorum sensing (Almasoud et al., 2023).

Several studies reported the antibacterial efficacy of *R. damascena* against foodborne pathogens, including *Staphylococcus aureus* (MIC = 0.28 mg/mL), *Listeria monocytogenes* (MIC = 0.37 mg/mL) and *Escherichia coli* (MIC = 0.42 mg/mL) (Sadeghi et al., 2021).

However, limited research explored its effects on beneficial lactic acid bacteria (LAB), which are essential for yoghurt and probiotic dairy fermentations. It is crucial to determine the minimum inhibitory concentration (MIC) of *R. damascena* extracts on LAB such as *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*, ensuring that its antimicrobial activity does not compromise yoghurt fermentation and probiotic viability (Dimitrova et al., 2019; Ahmad et al., 2022).

Given the increasing interest in natural preservatives and functional ingredients, this study aims to characterize bioactive compounds from spent rose petals (SRP), to evaluate the antioxidant activity, to investigate the antimicrobial property of the extract against common foodborne pathogens (*S. aureus*, *L. monocytogenes*, *E. coli*) and determine the minimum inhibitory concentration (MIC) of the extract on yoghurt and probiotic starter cultures. The findings from this research will provide valuable insights into the potential of *R. damascena* petal extracts as natural preservatives, supporting the development of functional dairy products with enhanced shelf life and health benefits.

MATERIALS AND METHODS

Materials

The lyophilized spent *Rosa damascena* Mill. petals extract (SRPE) was prepared by the procedure described by Vlahova-Vangelova et al. (2025). Reconstituted Dry Skim Milk (RDSM), with 9-10% dry matter and 0.1% fat content, was prepared and sterilised by heating at 121°C for 10 min.

Starter cultures

Direct Vat Set (DVS) yoghurt starter culture YF-3331 (Chr. Hansen) containing *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* strains, and probiotic yoghurt culture ABY-3 (Chr. Hansen) containning *Bifidobacterium* species, *Lactobacillus acidophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, and *Streptococcus thermophilus* strains were used.

Test microorganisms

Fifteen microorganism strains, including five Gram-positive bacteria (*Bacillus subtilis* ATCC 6633, *Bacillus cereus* NCTC 11145, *Staphylococcus aureus* ATCC 6538P, *Listeria monocytogenes* NBIMCC 8632, *Micrococcus luteus* 2YC_YT), five Gram-negative bacteria (*Salmonella enteritidis* ATCC 13076, *Klebsiella pneumoniae* ATCC 13883, *Escherichia coli* ATCC 25922, *Proteus vulgaris* ATCC 6380, *Pseudomonas aeruginosa* ATCC 9027), yeasts *Candida albicans* NBIMCC 74, and four fungi (*Aspergillus niger* ATCC 1015, *Fusarium oxysporum*, *Penicillium chrysogenum*, *Fusarium moniliforme* ATCC 38932) from the collection of the Department of Microbiology at the University of Food Technologies, Plovdiv, Bulgaria, were selected for the antimicrobial activity test.

Culture media

Luria-Bertani Agar Medium with Glucose (LBG Agar). LBG agar was used for cultivation of the test bacteria. A quantity of 50 g of LBG-solid substance mixture (containing 10 g tryptone, 5 g yeast extract, 10 g NaCl, 10 g glucose and 15 g agar) was dissolved in 1 L of deionized water, pH 7.5±0.2.

Malt Extract Agar (MEA). MEA was used for cultivation of the test yeasts and fungi. A quantity of 50 g of the MEA-solid substance mixture (containing 30 g malt extract, 5 g mycological peptone and 15 g agar) was dissolved in 1 L of deionized water, pH 5.4±0.2. The culture media were prepared according to the manufacturer's instructions (Scharlab SL, Barcelona, Spain) and autoclaved at 121°C for 20 min before use.

Methods

Sample preparation

The SRPE is dissolved in distilled water in proper concentration according to each of the following assays.

Total Polyphenol Content (TPC)

Total polyphenols were determined according to the method of Singleton and Rossi (1965) with

some modifications from Dinkova et al. (2022) using the Folin-Ciocalteu's reagent. Gallic acid was employed as a calibration standard and the results were expressed in mg Gallic acid equivalents (GAE) per 100 g.

HPLC Determination of Phenolic Compounds

HPLC analyses were performed on an UHPLC system Nexera-i LC2040C Plus (Shimadzu Corporation, Kyoto, Japan) with a UV-VIS detector and a binary pump. The column was Poroshell 120 EC-C18 (3 mm×100 mm, 2.7 μm), thermostated at 26°C. The flow rate was 0.3 mL/min and the injection volume was 5 μL. The derivatives were detected at λ = 280 nm. The mobile phase consisted of A: 0.5% acetic acid and B: 100% acetonitrile. The gradient condition started with 14% (B), between 6 and 30 min, linearly increased to 25% (B), and then to 50% (B) at 40 min. The identification of compounds was confirmed by a comparison of retention times utilizing standard solutions and standard calibration curves of different phenolic compounds.

HPLC Determination of Free Sugars

The analysis of free sugars was performed on a ZORBAX Carbohydrate (5 μm, 4.6 × 150 mm, Agilent) and a ZORBAX Reliance Cartridge guard column. An UHPLC system Nexera-i LC2040C Plus (Shimadzu Corporation, Kyoto, Japan) with a binary pump and a 20 A refractive index detector. Ten microliters of the extract were eluted at a flow rate of 0.6 mL/min at 30°C with a mobile phase composed of a mixture of acetonitrile and water (80:20 v/v). The concentration of sugars, detected by their retention time, in the sample was obtained using a calibration curve built by plotting the peak area against the concentrations of each standard.

HPLC Determination of Organic Acids

HPLC determination of organic acids was performed on an UHPLC system Nexera-i LC2040C Plus (Shimadzu Corporation, Kyoto, Japan) with a UV-VIS detector and a binary pump. Separation was performed on a Shimadzu Shim-pack GIST C18 (5 μm, 4.6×250 mm), Japan at 25°C. Twenty microliters of the extract, were injected and eluted (1.0 mL/min) isocratically with a 25 mM solution of K_2HPO_4 in water, whose pH was finely adjusted to 2.4 with H_3PO_4. The UV detector was set at 210 nm. The concentration of each organic acid in the sample was calculated using a calibration curve obtained by using five different concentrations for each acid. The peak corresponding to different acids was confirmed by a comparison of the retention time with that of the standards.

Antioxidant Activity

Determination of radical scavenging capacity.

The 1.1-diphenyl-2-picrylhydrazil radical (DPPH•) was used according to the method of Brand-Williams et al. (1995), with modifications from Dinkova et al. (2014). The radical scavenging capacity against the 2.2-azinobis 3-ethylbenzthiazoline-6-sulfonic acid radical ($ABTS^{\bullet+}$) was evaluated following the method of Shopska et al. (2022). Both radical scavenging capacities are presented as percent of inhibition by the SRPE solution - 100 mg/L.

Determination of transition metals-chelating activity.

The ferric (Fe^{3+}) reduction ability of plasma - FRAP assay was performed following the method of Benzie and Strain (1996) with modifications from Dinkova et al. (2014).

The cupric (Cu^{2+}) reducing antioxidant capacity - CUPRAC test is done by method of Shopska et al. (2022).

Oxygen radical absorbance capacity (**ORAC**) activity was measured on a microplate reader FLUOstar OPTIMA (BMG Labtech, Ortenberg, Germany) with excitation at λ = 485 nm and emission at λ = 520 nm, according to the method of Ou et al. (2001) with some modifications by Denev et al. (2010). Trolox was used for building the standard curve and results were expressed in micromole Trolox equivalents (μmol TE) per gram.

Hydroxyl radical averting capacity (**HORAC**) activity was determined with excitation at λ = 485 nm and emission at λ = 520 nm, according to Ou et al. (2002). Gallic acid was used for the standard curve and results were expressed in micromole gallic acid equivalents (μmol GAE) per gram.

Antimicrobial activity assay and Minimum inhibitory concentration (MIC)

The antimicrobial activity and minimum inhibitory concentration (MIC) of SRPE determined according to the methods previously described by Tumbarski et al. (2024).

Determination of minimum inhibitory concentration (MIC) and maximum concentration without inhibitory effect (MCWI)

The MIC and MCWI of the polyphenol extract on a DVS starter culture were determined by inoculating and coagulation of reconstituted dry milk (RDSM). The test samples were divided into two batches. The first batch was heated to 43°C and inoculated with YF-3331 starter culture according to the manufacturer's instructions (Chr. Hansen). The inoculated milk was distributed into eight test tubes. A solution of the polyphenol extract was prepared by dissolving lyophilized polyphenol extract in 10 mL of RDSM to obtain a final extract concentration of 10 mg/mL in milk (Tube 1). Serial dilutions were prepared from this stock solution as follows: Tube 1 was stirred and 5 ml were withdrawn and transferred to Tube 2. This serial transference was repeated until Tube 7. Tube 8 (containing only milk + starter culture) was used as a control. All tubes were then incubated at 43°C until coagulation of milk.

The second batch was heated to 37°C and inoculated with ABY-3 starter culture according to the manufacturer's instructions (Chr. Hansen). The procedure was the same as for the first batch. All tubes were incubated at 37°C until milk coagulation.

The maximum concentration of polyphenols without inhibitory effect (MCWI) was determined as the highest concentration of extract in which the growth of the lactic acid bacteria was not inhibited (i.e., milk coagulation still occurred).

Statistical analysis

Descriptive statistical analysis was performed using SPSS (Statistical Package for the Social Sciences) software (IMB, version 29.0, 2022).

RESULTS AND DISCUSSIONS

The concentration of total phenolic compounds (TPC) and the individual compounds are presented in Table 1. Our results for TPC confirm previously reported (Slavov et al., 2020; Vlahova-Vangelova et al., 2025). As previously reported by Slavov et al. (2020), Dragoev et al. (2021) and Charoimek et al. (2024) we also evaluated the presence of Gallic, Protocatechuic, p-Coumaric acids and Rutin.

Table 1. Phenolic composition of the SRPE

	TPC, mg GAE/g	Gallic acid, mg/g	Protocatechuic acid, mg/g	p-Coumaric acid, mg/g	Rutin, mg/g
SRPE	292.28 ±9.92	9.49 ±0.25	1.68 ±0.19	0.05 ±0.01	1.44 ±0.01

The SRPE contained small quantities of arabinose and fructose as presence of the natural sugars (Table 2). Slavov et al. (2017) reported a different quantity of both of the sugar depending on the extraction method.

Table 2. Neutral sugars of the SRPE

	Arabinose, g/100 g	Fructose, g/100 g
SRPE	4.7±0.11	5.0±0.15

In the solution of SRPE were found different organic acids such as oxalic, tartaric, malic and ascorbic (Table 3). Those results confirm what was reported by Halawani (2014).

Table 3. Organic acids composition of the SRPE

	Oxalic acid, mg/g	Tartaric acid, mg/g	Malic acid, mg/g	Ascorbic acid, mg/g
SRPE	4.43 ±0.04	51.40 ±1.73	24.63 ±0.06	11.54 ±0.10

The SRPE solution exhibited a strong radical scavenging activity against the DPPH• and ABTS•$^{+}$ (Figure 1).

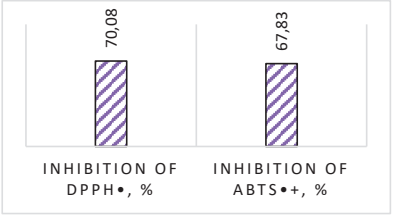


Figure 1. Radical scavenging activity of SRPE

The solution - 100 mg/L reduced up to 70% of the DPPH• radicals. Dina et al. (2021) also studied different extracts from spent *Rosa damascena* petals and their inhibitory activity was similar in the exact same concentration. Meanwhile, the inhibition of the ABTS•$^{+}$ radical was around 68%. Similar lower radical scavenging activity against the ABTS•$^{+}$ compared to the DPPH• was reported by others (Dudonne et al., 2009; Gulcin and Alwasel,

2023; Vlahova-Vangelova et al., 2025). In the meantime, Charoimek et al. (2024) report a strong inhibitory potential against both DPPH• and ABTS•$^{+}$ free radicals. Furthermore, measuring at different wavelengths can either introduce or eliminate colour interference from the sample (Aleixandre-Tudo et al., 2017).

Our findings support previous assertions that relying on a single methodology to assess antioxidant activity can sometimes lead to misleading results (Gulcin and Alwasel, 2023; Önder, 2023).

Table 4. Antioxidant activity of the SRPE

	FRAP, mM TE/g	CUPRAC, mM TE/g	ORAC, mM TE/g	HORAC, mM GAE/g
SRPE	4022.66 ±0.98	1858.33 ±3.41	4583.20 ±2.83	2479.40 ±3.22

The solution of SRPE exhibited strong iron-reducing potential - FRAP (Table 4). Our results are similar to reported by Slavov et al. (2020), Vlahova-Vangelova et al. (2025) and Dragoev et al. (2021).

The evaluated cupric (Cu^{2+}) reducing antioxidant capacity - CUPRAC of SRPE was significantly lower compared to the FRAP, yet still sufficient. Others are reporting that the extract from spent rose petals posses a strong reduction ability against cupric ions (Petkova et al., 2020; Vlahova-Vangelova et al., 2025). In the meantime, FRAP and CUPRAC values were higher that those reported by Petkova et al. (2021) for microwave-assisted extraction.

Oxygen radical absorbance capacity (ORAC) of SRPE was significant (Table 4) and comparable to the presented by others (Dudonne et al., 2009; Slavov et al., 2017 and 2020). Meanwhile the hydroxyl radical averting capacity (HORAC) was 2479.40±3.22 mM GAE/g. Our results are comparable with the reported antioxidant activity of ethanolic extracts of waste rose flowers by Slavov et al. (2017) and the microwave-assisted extracts of Petkova et al. (2021).

All of the found phenolic compounds are well-known for their antioxidant activity against free radicals like DPPH• and ABTS•$^{+}$ (Figure 1), also chelation of transition metals as ferric and cupric ions (Table 4), confirmed by our results.

The results from the antimicrobial activity screening (Table 5) demonstrated that the SRPE possessed the most significant inhibitory effect on *M. luteus* 2YC_YT and *C. albicans* NBIMCC 74 (MIC = 0.313 mg/mL), followed by *L. monocytogenes* NBIMCC 8632 and *P. vulgaris* ATCC 6380 (MIC = 0.625 mg/mL). The inhibitory activity against *B. subtilis* ATCC 6633, *B. cereus* NCTC 11145, *S. aureus* ATCC 6538P, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 9027, and fungi *F. oxysporum* and *P. chrysogenum* was moderate to low, with MIC values > 1.25 mg/mL. The SRPE did not inhibit the growth of test microorganisms *S. enteritidis* ATCC 13076, *K. pneumoniae* ATCC 13883, and fungi *A. niger* ATCC 1015 and *F. moniliforme* ATCC 38932. The obtained results revealed the promising antimicrobial potential of the studied rose extract for further application as a valuable ingredient in food products and cosmetic formulations.

Table 5. Antimicrobial activity and MIC values of SRPE

Test microorganisms	MIC, mg/mL
B. subtilis ATCC 6633	5
B. cereus NCTC 11145	5
S. aureus ATCC 6538P	10
L. monocytogenes NBIMCC 8632	0.625
M. luteus 2YC_YT	0.313
E. coli ATCC 25922	5
P. vulgaris ATCC 6380	0.625
P. aeruginosa ATCC 9027	5
C. albicans NBIMCC 74	0.313
F. oxysporum	1.25
P. chrysogenum	2.5

As sources of biologically active compounds with antimicrobial effect, *R. damascena* extracts have been investigated in numerous studies. Chroho et al. (2022) examined the biological activity of *R. damascena* from Morocco and stated that the hydroethanolic flower extract exhibited an antimicrobial effect on *E. coli* (MIC = 20.83 mg/mL), *S. typhimurium* (MIC = 41.66 mg/mL), *S. aureus* (MIC = 20.83 mg/mL), and *L. monocytogenes* (MIC = 20.83 mg/mL), witch values were lower as compared to ours. These results confirmed that the *R. damascena* flowers might be used as natural antimicrobial agents in the cosmetic and pharmaceutical sectors. In a study conducted by Trendafilova et al. (2023), the extract from *R. damascena* flowers from Bulgaria, obtained by aqueous-ethanolic

extraction and its phenolic-enriched fraction, obtained by re-extraction with ethyl acetate, exhibited significant inhibitory activity against *Propionibacterium acnes*, *Staphylococcus epidermidis* ATCC 12228 and *S. aureus* ATCC 25923, and lower inhibitory activity against *B. cereus* ATCC 11778, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27583; however in contrast to our results, both extracts did not inhibit the yeasts *C. albicans* ATCC10231. Jafari-Sales et al. (2020) investigated a methanolic extract obtained from petals of *R. damascena* from Iran. The authors observed that the extract was more effective against Gram-positive bacteria than Gram-negative ones, with a diameter of the inhibition zones against *S. aureus* of 26.2 mm and *B. cereus* of 24.6 mm at the extract's concentration of 400 mg/mL. MIC values of the methanolic extract were 6.25 mg/mL (*S. aureus*), 12.5 mg/mL (*B. cereus*), 50 mg/mL (*E. coli*), and 100 mg/mL (*P. aeruginosa*), respectively. Considering the antimicrobial effect of the studied *R. damascena* extract, the authors concluded that it can be used as an alternative to conventional chemical drugs in the treatment of the infections caused by these microorganisms.

The antimicrobial potential of spent *Rosa damascena* petals extract (SRPE) was evaluated against Direct Vat Set (DVS) starter cultures YF-3331 and ABY-3 (Table 6), which are commonly used in yoghurt production. The minimum inhibitory concentration (MIC) and maximum concentration of extract without inhibitory effect (MCWI) were determined to assess the suitability of incorporating SRPE into yoghurt formulations without negative impact on fermentation.

Table 6. Antimicrobial activity of SRPE on DVS starter culture

SRPE, mg/mL	Starter culture	
	YF-3331	ABY-3
10	-	-
5	-	-
2.5	-	-
1.25	-	-
0.625	-	-
0.313	+	-
0.156	+	+
0 (control)	+	+

- Non coagulation; + Coagulation.

The MIC values represent the lowest concentration of SRPE that completely inhibits the growth of the starter cultures. In this study, the MIC for YF-3331 was found to be 0.625 mg/mL, while for ABY-3, the MIC was 0.313 mg/mL (Table 7). This suggests that ABY-3 was more sensitive to the antimicrobial effect of SRPE, which could be attributed to the higher polyphenolic content in the extract (Table 1), particularly compounds such as Gallic acid, Protocatechuic acid, and p-Coumaric acid (Shang et al., 2019). These compounds are known to interfere with bacterial cell membranes and inhibit enzyme functions, leading to antimicrobial activity (Bora et al., 2020).

Table 7. Minimum inhibitory concentration (MIC) and maximum concentration of polyphenols without inhibitory effect (MCWI)

Starter culture	MIC	MCWI
YF-3331	0.625 mg/mL	0.313 mg/mL
ABY-3	0.313 mg/mL	0.156 mg/mL

The MCWI refers to the highest concentration of SRPE that can be included in the fermentation process without inhibiting the growth of LAB. For the YF-3331 culture, the MCWI was found to be 0.313 mg/mL, and for ABY-3, it was 0.156 mg/mL. These data are in agreement with the results obtained from other authors (Dimitrova et al., 2019). The findings in the present study indicate that higher concentrations of SRPE could inhibit the starter cultures and affect yoghurt fermentation, particularly with the ABY-3 culture. As polyphenols are known for their antioxidant activity, it is possible that their oxidative stress on microbial cells could contribute to the observed antimicrobial effects (Mahmood et al., 2020).

CONCLUSIONS

The spent *Rosa damascena* petals extract (SRPE) exhibited strong antioxidant activity, effectively scavenging DPPH• and ABTS•+ radicals, with notable FRAP and CUPRAC values. The presence of phenolic compounds like Gallic acid and Rutin, along with organic acids such as ascorbic acid, contributed to its antioxidant and metal-chelating properties. These findings confirm SRPE as a potent natural antioxidant, with potential applications in foods.

The antimicrobial activity of SRPE is a promising characteristic for its use as a natural preservative in yogurt production, helping to extend shelf life and control pathogen contamination. However, for successful incorporation, SRPE must be carefully dosed to ensure probiotic viability and maintain fermentation efficiency. These findings confirm SRPE as a potent natural antioxidant, with potential applications in foods.

ACKNOWLEDGEMENTS

This research was financially supported by projects KP-06-Slovakia/7 from 13.08.2024 and APVV-SK-BG-23-0002 (grant 27A4-A-64039).

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

EVALUATION OF THE INHIBITORY EFFECT OF ESSENTIAL OILS AGAINST *Fusarium sporotrichioides* ISOLATED FOR THE FIRST TIME FROM STRAWBERRY PLANTS IN ROMANIA

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Abstract

This study evaluated the in vitro inhibitory effects of essential oils (EO) on Fusarium sporotrichioides isolated for the first time from strawberry plants in Romania. Oregano and cinnamon EO exhibited strong and consistent inhibitory activity, achieving complete fungal inhibition at both high (500 and 1000 µl/l) and moderate (125 µl/l for oregano and 250 µl/l for cinnamon) dosed. Clove EO revealed to be effective only in higher doses (500 and 1000 µl/l) and showed reduced efficacy at lower dosage (35-125 µl/l) compared to oregano and cinnamon EO. In contrast, lemon, mint, and tea tree EO demonstrated limited and inconsistent fungal inhibition. Lemon EO intensified mycelial pigmentation without affecting the growth. Mint EO reduced the mycelial density but did not hinder radial expansion, while tea tree oil had minimal inhibitory impact, with only 17.96% inhibition at 1000 µl/l after 6 days of incubation. These findings highlight the potential use of some specific EO to control F. sporotrichioides, as oregano and cinnamon EO showed high fungal inhibitory efficacy, even at lower doses.

Key words*: biocontrol, essential oils, fungal inhibition, Fusarium sporotrichioides, strawberry.*

INTRODUCTION

Strawberry production in Romania has seen rapid growth, driven by favorable climate and soil conditions across various regions. These conditions make strawberries a highly adaptable crop, suitable for a variety of soil types and microclimates. Romania has considerable potential for high strawberry productivity, with average yields often exceeding 20 tons per hectare (Soare & Chiurciu, 2021). Projections suggest further growth, with an estimated output of approximately 25,270 metric tons by 2026, fueled by expanding cultivated areas and advancements in agricultural practices (ReportLinker, n.d.). However, despite these favorable prospects, strawberry cultivation is increasingly threatened by *Fusarium* wilt, primarily caused by *Fusarium oxysporum* f. sp. *fragariae* (Fof), which leads to significant economic losses globally, affecting both yield and quality (Fang et al., 2012; Koike & Gordon, 2015).

The management of *Fusarium* wilt presents a significant challenge due to the soil-borne nature of the pathogen and the restrictions on chemical fumigant use (Koike & Gordon, 2015). Although some chemical fungicides, such as tebuconazole, have been employed, the increasing issue of reduced sensitivity among certain *Fusarium* spp. strains has limited their effectiveness (Cho & Kwak, 2022). Recent studies have evaluated the efficacy of hydrogen peroxide and azoxystrobin treatments in controlling *Fusarium* spp. in strawberry crops, highlighting their potential role in disease management (Țane Lumînare & Cristea, 2024). However, the long-term effectiveness of such treatments and their impact on pathogen resistance remain concerns, emphasizing the need for alternative, sustainable disease management strategies.

Biological control agents, including essential oils (EOs), have gained increasing attention as eco-friendly alternatives to synthetic fungicides. Previous studies have demonstrated the

antifungal properties of EOs, including those of cinnamon, oregano, and thyme, which inhibit the growth of *Fusarium oxysporum* in a dose-dependent manner (Park et al., 2017; Massoud et al., 2020; Stan Tudora et al., 2022). Other plant-derived EOs have also shown strong antifungal activity against various *Fusarium* species, supporting their potential use in disease management. For example, *Mentha arvensis* EO has been shown to inhibit the growth of *F. proliferatum* and *F. verticillioides* (Kumar et al., 2016), whereas savory and thyme EOs have demonstrated significant antifungal activity against *Fusarium oxysporum* f. sp. *basilici* (Lopez-Reyes et al., 2016).

The use of plant-based biopesticides and natural extracts in plant protection has also been explored, with research highlighting their antimicrobial and phytostimulatory effects (Ichim et al., 2017; Perișoara et al., 2022). For instance, extracts from *Tagetes erecta* have been reported to exhibit synergistic antipathogenic effects in the presence of rhizobacteria (Perișoara et al., 2022), while fenugreek extracts have been studied for their influence on seed germination and plant health (Perișoara et al., 2023). Additionally, biofungicidal prototypes have shown promising *in vitro* antifungal activity against *Fusarium* spp. (Cristea et al., 2024). These findings support the increasing interest in developing plant-based alternatives to chemical treatments.

Furthermore, EOs from *Bursera morelensis* and *Lippia graveolens*, particularly rich in carvacrol, have shown potent antifungal effects on *Fusarium* species by affecting hyphal morphology, spore production, and germination (Medina-Romero et al., 2022). The efficacy of EOs has been demonstrated in multiple studies, with cumin, basil, and geranium EOs significantly reducing root rot caused by various *Fusarium* species in cumin plants under both greenhouse and field conditions (Hashem et al., 2010). However, in certain cases, such as with basil seeds, pathogen control provided by heat treatments combined with biocontrol agents and chemical fungicides did not perform as well as chemical fungicides (Lopez-Reyes et al., 2016). The antifungal activity of EOs is largely attributed to their chemical composition, particularly the presence of oxygenated compounds, which disrupt fungal cell functions (Kumar et al., 2014; Roselló et al., 2015). Despite their promising potential, the effectiveness of EOs can vary depending on the targeted *Fusarium* species and the specific bioactive constituents of the oils used. This variability makes EOs a promising alternative to chemical pesticides for controlling *Fusarium* infections in crops (Samie, 2012; Fontana et al., 2020; Radice et al., 2022).

Although the antifungal potential of EOs is well documented, limited research exists regarding their effects on *F. sporotrichioides*, particularly in strawberry plants. This study aims to address this gap by evaluating the inhibitory effects of selected EOs on *F. sporotrichioides* isolated for the first time from strawberry plants grown in Romania. By exploring these eco-friendly alternatives, we aim to provide valuable insights into their potential role in integrated disease management strategies for strawberry cultivation. Our findings could provide a foundation for sustainable solutions to combat *Fusarium*-related diseases, reduce dependence on chemical treatments, and minimize environmental impacts.

MATERIALS AND METHODS

Pathogen isolation and identification

Strawberry plant samples of the Alba cultivar, expressing stunting and leaf wilting symptoms, were collected from a commercial strawberry plantation in Giurgiu County. The sampling focused on crown areas exhibiting visible symptoms of fusariosis to ensure that the material was representative. The samples were handled under sterile conditions to prevent contamination and promptly transported to the laboratory for subsequent analysis. Disinfection involved a four-step protocol: (1) washing with sterile distilled water for 1 min to remove surface debris, (2) immersion in 70% ethanol for 1 min to eliminate surface contaminants, (3) treatment with 4% sodium hypochlorite for 2 min for thorough disinfection, and (4) three subsequent rinses with sterile distilled water, each lasting 1 min. The disinfected material was dried in a laminar flow hood to prevent additional contamination. Using a sterile scalpel, the plant material was cut into small fragments (approximately 0.5 cm), which were then placed in sterile Petri dishes containing

potato dextrose agar (PDA) medium. The plates were incubated at 25°C in darkness, for five days, to promote fungal growth. After incubation, the individual fungal colonies were transferred to fresh PDA medium to obtain pure cultures. The initial identification of fungal isolates was based on macroscopic colony characteristics such as color, texture, and growth pattern, and microscopic examination of reproductive structures using an optical microscope (Nikon Eclipse 2000 LED MV R, Nikon Corporation, Tokyo, Japan). To confirm species identity, DNA sequencing was carried out, targeting the ITS1-5.8S-ITS2 region. Fungal DNA was extracted from fresh pure culture using the ZR Fungal/ Bacterial MiniPrep™ commercial kit (ZymoResearch, SUA). The ITS-5.8S region was amplified using the primers set ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3'). The PCR was performed in 50 µl reaction volume, containing 1X Green Buffer with magnesium chloride, 0.2 mM dNTPs, 0.5 µM of each primers, 0.2 U of DreamTaq DNA Polymerase (Thermo-Scientific, Baltics, UAB, Vilnius, Lithuania), and 10 ng of template DNA, in sterile MilliQ water (Mohamed et al., 2024). The PCR program included an initial step of 4 min at 94°C for DNA denaturation, followed by 35 cycles of denaturation at 94°C 1 min, primers' annealing at 45°C for 1 min, and elongation at 72°C for 2 min, followed by a step of 10 min at 72°C for final elongation 10 min. The amplified product was verified through agarose gel electrophoresis, and the PCR products was sent for purification and DNA sequencing at CeMIA (Cellular and Molecular Immunological Applications, Greece). The resulting forward and reverse sequences were assembled with BioEdit software and submitted to the NBLAST program to be compared to similar DNA sequences found in the National Center for Biotechnology Information (NCBI) database. The ITS-5.8S partial sequence of the identified fungal isolate was then submitted to NCBI GenBank.

Evaluation of essential oils antifungal activity

To determine essential oils (Eos) antifungal activity against *Fusarium* sp., six EOs were tested, oregano, clove, mint, cinnamon, tea tree, and lemon. These EOs were purchased from ELLEMENTAL, a certified supplier, that ensure their high quality and purity. Stock solutions of each EO were prepared by mixing the oils with Tween 80 as an emulsifier in a 2:1 (v/v) ratio to enhance dispersion in the culture medium. Six concentrations (35, 65, 125, 250, 500, and 1000 µl/l) were prepared for each EO, and *in vitro* treatments were conducted in triplicate, to ensure reliable results. The emulsified oil mixtures were then incorporated into a molten PDA medium before solidification to achieve uniform distribution. The media were aseptically purred in 9 cm diameter sterile Petri plates. PDA medium was used as control. Mycelial discs, 0.5 cm diameter, were aseptically cut from the edge of 7-day-old *Fusarium* colonies and placed in the center of Petri dishes. The plates were incubated at 25°C, and colony diameters were measured in two perpendicular directions after 3, 6, 8, 11, and 15 days post-inoculation. The average diameter was used to calculate mycelial growth inhibition (MGI) using the formula: $MGI\ (\%) = (C - T)/C \times 100$, where C is the mean colony diameter in the control group and T is the mean colony diameter in the treatment group (Riccioni & Orzali, 2011). The IC50 and IC90 values, representing the concentrations required to inhibit 50% and 90% of mycelial growth, respectively, were calculated using regression analysis.

Data Analysis

Statistical analyses were conducted using the R software to compare the antifungal effects of EOs and their concentrations over time. ANOVA tests were used to determine whether there were significant differences, and Tukey's HSD tests were used to pinpoint where these differences occurred. A significance level of $p < 0.05$ was used for all analyses.

RESULTS AND DISCUSSIONS

Fungal macroscopic appearance

Symptomatic strawberry plants revealed a fungal pathogenic attack. The isolated phytopathogen was identified as *Fusarium* sp., based on macroscopic morphological appearance. Isolated colonies exhibited rapid growth on PDA medium, reaching a diameter of 7-9 cm within approximately 7 days at 25°C. In the early growth stages, the mycelium was dense and

white, with a slight violet hue. As the colonies matured, the color transitioned to pink and reddish-brown, becoming more pronounced in the central region. The texture of the colonies was fluffy, and the mycelial filaments extended radially, forming a uniform and well-defined pattern. Upon maturation, the color intensified, and the pigment diffused into the culture medium, creating a pinkish or orange tint in the basal layer. The colony margins remained well-defined, with a compact and consistent structure across the surface of the medium.

Microscopic characteristics

Microscopic examination confirmed the presence of characteristic fusiform macroconidia with 3 to 5 septa and curved apical cells, along with pyriform microconidia and globose chlamydospores. The macroconidia were generally grouped in sporodochia, whereas the chlamydospores were observed either solitarily or in chains, displaying thick walls. These features align with previous descriptions and ensure accurate identification of *Fusarium* sp., showing high morphological correspondence to *F. sporotrichioides*, as described in the literature (Ivanová et al., 2016; Abdelmagid et al., 2021).

Molecular Identification

The isolated strain, encoded FusCL, was identified at species level based on DNA sequence analysis of the ITS1-5.8S-ITS2 region. The internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence of this strain have 530 bp in length, and are available on NCBI under the GenBank no. PQ675606.1. Molecular comparison of the FusCL strain with other highly similar DNA sequences from NCBI database revealed 100% identity with *F. sporotrichioides* ATCC MYA-3963 (accession number FJ614637.1) with 98% query coverage. This genetic identification supports the morphological and microscopic observations, confirming that the isolated strain belongs to *Fusarium sporotrichioides* species.

Fungal inhibition using EOs

Tested EOs demonstrated varying degrees of inhibitory effects on *Fusarium sporotrichioides* FusCL, with notable differences based on the oils type, concentration and exposure time (Figure 1).

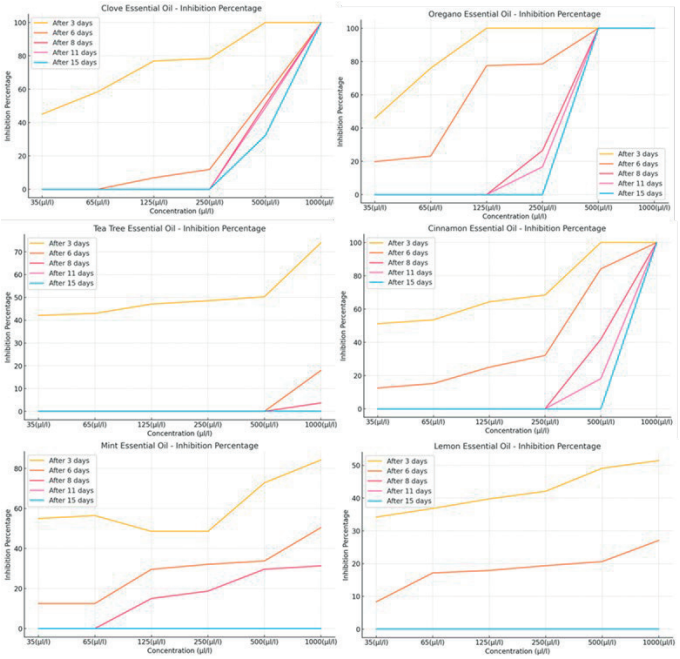


Figure 1. Inhibition percentages of *Fusarium sporotrichioides* induced by different concentrations of essential oils over time

Oregano EO exhibited the most consistent and potent inhibitory effects. At concentrations of 125 µl/l and higher, complete inhibition (100%) was observed as early as day 3, and this effect was maintained through day 15 at the highest concentrations (500 µl/l and 1000 µl/l). In contrast, lower concentrations (35 and 65 µl/l) showed a gradual decline in inhibition over time, with no effect observed by day 15. Lemon EO had a moderate initial inhibitory effect, with percentages ranging from 34.21% to 51.46% on day 3. However, its efficacy diminished rapidly, and no inhibition was observed beyond day 6, regardless of concentration. Cinnamon EO displayed strong inhibition at high concentrations, achieving complete inhibition (100%) at 500 µL/l and 1000 µL/l by day 3, and sustained this effect at 1000 µL/l through day 15. Lower concentrations exhibited a gradual reduction in inhibition, with no effect observed after day 8 at 35 µl/l and 65 µl/l. Mint EO initially showed significant inhibition, particularly at 500 µl/l and 1000 µl/l, with percentages of 72.81% and 84.21%, respectively, on day 3. However, the inhibitory effect was inconsistent, and all concentrations lost their efficacy by day 11. Tea tree EO exhibited the lowest overall efficacy, achieving a maximum inhibition of 73.98% at 1000 µl/l after day 3. The effect decreased rapidly, with minimal inhibition observed by day 8, and no inhibition detected by day 11 or beyond. Clove EO demonstrated a significant inhibitory effect, achieving complete inhibition (100%) at 1000 µl/l after 6 days, and sustained this effect through day 15. At 500 µL/l, inhibition remained substantial, but gradually decreased over time. The lower concentrations exhibited limited and short-lived effects.

IC50 and IC90 values

Oregano EO demonstrated the highest efficacy, with IC50 values ranging from 309.31 µl/l to 449.80 µl/l and IC90 values between 312.77 µl/l and 453.13 µl/l. An intermediate efficacy was obtained with the cinnamon EO, which exhibited IC50 values ranging from 426.96 µl/l to 667.13 µl/l and IC90 values between 430.42 µl/l and 670.33 µl/l. Clove EO showed the lowest efficacy among the three, with IC50 values ranging from 403.49 µl/l to 501.49 µl/l and IC90 values between 406.95 µl/l and 505.90 µl/l (Figure 2).

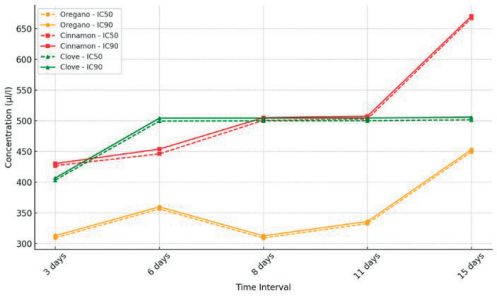


Figure 2. Concentrations required to inhibit 50% (IC50) and 90% (IC90) of mycelial growth with oregano, cinnamon, and clove essential oils over time

The other essential oils tested (lemon, mint, and tea tree) did not exhibit sufficiently high inhibition percentages to allow for the calculation of these two indicators.

Statistical analysis: ANOVA and Post-Hoc Tests

During the first three days of the experiment, the clove, mint, cinnamon, and oregano EOs exhibited significant differences in inhibiting mycelial growth ($p < 0.001$), indicating a rapid and potent antifungal effect. A direct relationship between concentration and inhibition efficacy was observed ($p < 0.001$), underscoring the importance of an adequate dose to maximize the effect. By day 6, the differences among the EOs remained significant ($p < 0.001$), confirming the short-term consistency of their effects.

On day 8, the differences between the EOs were no longer significant ($p = 0.068$), suggesting a stabilization of the individual antifungal effects. However, concentration continued to have a significant influence ($p = 0.003$), emphasizing the critical role of dosage in maintaining long-term efficacy. Throughout the entire observation period, oregano and clove EOs maintained a pronounced antifungal effect, particularly at higher concentrations. By days 11 and 15, differences between oils became nonsignificant ($p = 0.091$ and $p = 0.182$, respectively), but the significant impact of concentration persisted ($p = 0.002$ and $p = 0.003$), highlighting the importance of dosage as a critical factor in the effective management of the pathogen.

The ANOVA and post-hoc Tukey HSD tests confirmed that, although the differences among EOs were more pronounced in the early days, the long-term efficacy was significant for oregano and clove EOs. This finding underscores the necessity of an appropriate dosing strategy to sustain durable and effective antifungal control against *F. sporotrichioides*. The results of the statistical analysis are summarized in Table 1.

EOs effects on *F. sporotrichioides* mycelium

The experiments revealed distinct effects of the EOs on the fungal color and density of the mycelium, which varied depending on the EOs concentration used (Figure 3).

Cinnamon and oregano EOs caused noticeable depigmentation of the mycelium, indicating significant metabolic stress, particularly at higher concentrations. This depigmentation effect was observed across all concentrations of clove and tea tree EOs used. However, unlike the tea tree EO, which did not inhibit mycelial growth, the clove EO exhibited a pronounced inhibition of growth at higher concentrations.

Another important observation was related to the mint EO, where the pink coloration of the mycelium persisted, but the density significantly decreased when increasing the EO concentration. This suggests a partial inhibition of hyphal formation, though radial expansion of the mycelium remained unaffected. Conversely, the lemon EO intensified the pink coloration but neither inhibited mycelial growth nor affected its density.

These observations highlight the complex interactions between the bioactive compounds present in the EOs and the fungal metabolism, influencing both the metabolic processes and the physical structure of the mycelium.

Table 1. Summary of statistical analysis results (ANOVA and Post-Hoc Tests)

Incubation time	EOs showing significant differences	Concentrations with significant differences	EOs	Concentrations	Significance
			p-Values ANOVA		
3 days	Clove, Mint, Cinnamon, Oregano	All concentrations are significant ($p < 0.001$)	< 0.001	< 0.001	***
6 days	Clove, Mint, Cinnamon, Oregano	All concentrations are significant ($p < 0.001$)	< 0.001	< 0.001	***
8 days	No significant differences between the EOs	Concentrations are significant ($p = 0.003$)	0.068	0.003	*(concentrations)
11 days	No significant differences between the EOs	Concentrations are significant ($p = 0.002$)	0.091	0.002	**(concentrations)
15 days	No significant differences between the EOs	Concentrations are significant ($p = 0.003$)	0.182	0.003	* (concentrations)

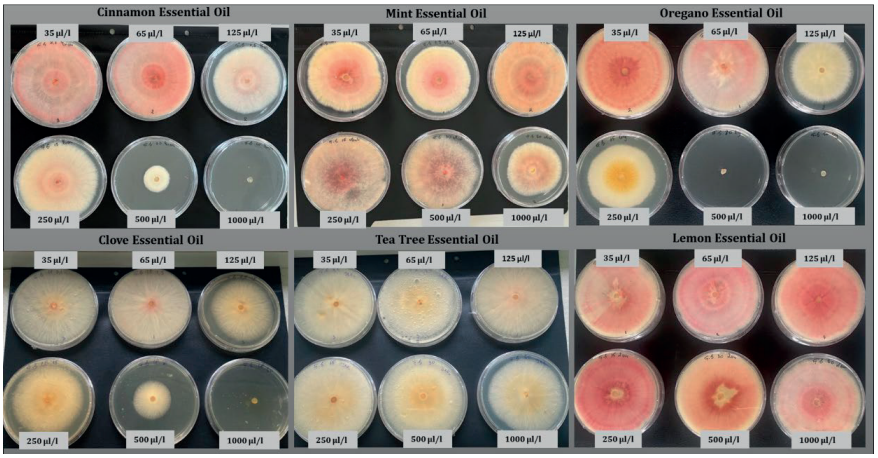


Figure 3. EOs effects in different concentrations on the growth of *F. sporotrichioides*

Our study evaluated the inhibitory effects of various EOs on *F. sporotrichioides*, isolated for the first time from strawberry plants in Romania. Tested EOs revealed significant variation in antifungal efficacy depending on the EO type, concentration, and exposure duration.

Oregano EO demonstrated the most consistent inhibitory effect, with complete inhibition (100%) of the fungal growth observed at concentrations as low as 125 µl/l by day 3, and up to day 15 at higher concentrations (500 µl/l and 1000 µl/l). These results are in accordance to with those of Střelková et al. (2021), who reported the potent antifungal activity of oregano EO against various *Fusarium* spp. Previous studies by Peschiutta et al. (2016) and Harčárová et al. (2021) attributed this efficacy to the high concentrations of carvacrol and thymol, which disrupt fungal cell membranes. This consistency across studies supports oregano EO as a promising natural antifungal agent.

Clove EO also exhibited substantial antifungal effects, with complete fungal inhibition at 1000 µl/l sustained for 15 days. Similarly, Sharma et al. (2016) and Irshad et al. (2023) reported clove EO with significant antifungal activity, emphasizing the role of eugenol in distorting fungal spores and disrupting cell membranes (Rana et al., 2011). The high eugenol content of clove oil makes it an effective alternative to synthetic fungicides for controlling *Fusarium* infections.

Cinnamon EO showed strong inhibition at high concentrations, achieving complete inhibition at 500 µl/l and 1000 µl/l by day 3, with sustained effects at 1000 µl/l through day 15. Roselló et al. (2018) and Lee et al. (2020) also highlighted the efficacy of cinnamon EO against *Fusarium* species, which is attributed to the ability of cinnamaldehyde to disrupt fungal cell walls and interfere with tubulin polymerization (Shahina et al., 2018). Our findings reinforce the potent antifungal potential of cinnamon EO and suggest its practical application in integrated disease-management strategies.

Mint EO initially exhibited significant inhibitory effects, achieving 72.81% and 84.21% inhibition at 500 µl/l and 1000 µl/l, respectively, on day 3. However, this efficacy diminished over time, with no inhibition observed on day 11. Similar observations were made by Sreenivasa et al. (2011) and Rachitha et al. (2017), who found that peppermint EO antifungal activity decreases as volatile components dissipate. The morphological changes in the hyphae observed in other studies (Rachitha et al., 2017) may partially explain the transient nature of mint oil's efficacy.

Tea tree EO exhibited moderate initial antifungal activity, with inhibition of 73.98% at 1000 µl/l on day 3, but the effect declined rapidly, with no inhibition detected on day 11. Previous studies, such as those by Sahab et al. (2014) and Paramalingam et al. (2023), demonstrated the antifungal properties of tea tree EO against various *Fusarium* spp., although its efficacy can vary based on fungal strain and environmental conditions. The rapid evaporation and degradation of tea tree EO likely contribute to the limited duration of its inhibitory effect.

Lemon EO showed the weakest overall efficacy, with an initial moderate inhibitory effect (34.21% to 51.46% on day 3) that quickly diminished, with no inhibition observed beyond day 6. Homa et al. (2015) and Palfi et al. (2019) similarly reported that lemon EO has a lower antifungal effectiveness than other tested EOs. The relatively weak potency of lemon oil may limit its use as a standalone treatment; however, it could have potential in combination strategies.

This study presents the first report of *F. sporotrichioides* on strawberry plants in Romania. Although this pathogen is primarily known to infect cereals such as wheat and corn (Beccari et al., 2018; Xue et al., 2019; Wang et al., 2020), research has demonstrated its ability to infect other plants, including soybean, peach, and pine (Ivanová et al., 2016; Khan et al., 2022; Xu et al., 2024). The significance of *F. sporotrichioides* identification on strawberries is highly valuable due to its potential production of harmful mycotoxins, such as T-2 and HT-2 (Meneely et al., 2023; Svoboda et al., 2024), which have implications on food safety. Future research should focus on testing fungal pathogenicity of *F. sporotrichioides* on strawberry plants to better understand the impact of the attack and to assess the risk of mycotoxin contamination.

Our findings emphasize the potential of EOs, particularly oregano, clove, and cinnamon, as natural antifungal agents against *F. sporotrichioides*. These EOs could offer

sustainable alternatives to chemical fungicides. However, further studies are needed to optimize their application methods and evaluate their efficacy under field conditions.

CONCLUSIONS

This study marks the first report of *Fusarium sporotrichioides* on strawberry plants in Romania, confirmed through both microbiologic and molecular analyses. These findings are significant, given the pathogen's known ability to produce harmful mycotoxins and their potential impact on food safety.

This study also explored the antifungal potential of various EOs, such as cinnamon, oregano, and clove, demonstrating the strong inhibitory effects against *F. sporotrichioides*, particularly at concentrations of 500 and 1000 µl/l. These EOs not only inhibited the radial growth of fungal colonies, but also disrupted the mycelial structure, suggesting a significant impact on the physiological processes of the pathogen. In contrast, lemon, mint, and tea tree EOs exhibited lower and inconsistent efficacies, most probably imposing higher doses to achieve substantial effects.

Overall, this study provides foundational insights into the presence and potential threat of *F. sporotrichioides* on strawberry crops and highlights the potential of EOs as eco-friendly biocontrol alternatives. However, further research, including field trials, is essential to validate these findings and fully understand their implications for sustainable disease management. The integration of natural antifungal solutions such as essential oils could play a crucial role in advancing sustainable agricultural practices and enhancing plant health management.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

EXPERIMENTAL RESEARCH FOR THE PROMOTION OF TECHNICAL EQUIPMENT FOR CLEARING MOUNDS AND FERTILIZING SOILS OCCUPIED BY GRASSLANDS

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Abstract

Depending on pedoclimatic and operational conditions, different situations may arise regarding the coverage of soils occupied by grasslands with mounds, stones, low-value vegetation, manure, etc. Based on the degree of coverage with mounds, low-value herbaceous and woody vegetation, manure, etc. and the size of these elements, different technological variants are selected for mechanizing the operations of clearing mounds, removing manure, leveling micro-relief, aerating, and fertilizing the soils occupied by grasslands. This paper presents the results of experimental research conducted under operational conditions of technical equipment designed for clearing mounds and fertilizing grassland soils. The experimental results obtained in this study are used to optimize the working process, aiming to surpass the performance of existing equipment in the same category. The goal is to provide farmers with access to technical equipment that meets the requirements for adapting techniques and technologies for the rational utilization of grasslands in the context of global and regional climate change.

Key words*: clearing weeds, fertilizing soils, grasslands.*

INTRODUCTION

Grasslands represent one of Earth's dominant biomes, covering approximately 30-40% of the terrestrial surface. They are essential for global ecological balance as they are characterized by the predominant presence of grasses, other herbaceous plants, various assemblages of other plant life forms, and diverse animal communities that contribute to biodiversity (Petermann & Buzhdygan, 2021).

Due to inefficient management, whether through overgrazing, intensive agriculture, or abandonment, grassland ecosystems are under enormous pressure, threatening biodiversity (Prangel et al., 2023).

Being among the most vulnerable ecosystems to climate change and human interventions, grasslands pose substantial political challenges in maintaining ecological balance at a global level (Zhang et al., 2024).

In Europe, natural grasslands, which cover limited areas and are increasingly fragmented, face unique conservation challenges due to agricultural development, urbanization, and anthropogenic climate change (Shipley et al., 2024).

The differentiated application of improvement measures for permanent grasslands requires a detailed understanding of vegetation characteristics, site conditions (soil, climate, relief factors), and land use practices (grazing, mowing, etc.), enabling sustainable management that increases productivity without compromising biodiversity (Liu & Li, 2023).

For example, fertilization increases the abundance of plant species with high average productivity indicator values and raises the average values of moisture indicators (Boch et al., 2021).

Additionally, through the application of mineral and organic fertilization, subalpine grasslands dominated by *Nardus stricta* can be improved, while the evaluation of green fodder mass production and pastoral value can be conducted based on floristic surveys, followed by the application of the forage-to-milk conversion coefficient (Maruşca, 2024).

An essential role, regardless of the practice used for improving permanent grasslands, is played

by grazing, which can have numerous positive effects on biodiversity if carried out properly and sustainably. It contributes to maintaining and even enhancing species diversity among plants and animals, positively influencing grassland ecosystems (Janišová et al., 2023).
Another crucial factor in increasing productivity and improving the efficiency of grasslands is the type of fertilization equipment used.
The application of fertilizers (organic or mineral) through uniform distribution - avoiding over-fertilization in certain areas and under-fertilization in others - using modern equipment stimulates biomass growth and improves forage quality (Mocanu et al., 2021).
The growing need to produce more food for an increasing population has led to the intensive use of grasslands, negatively affecting soil quality, biodiversity, and long-term productivity.
The solution does not lie in excessive exploitation, but rather in sustainable management through modern technologies to maintain a balance between production and ecosystem conservation (Wang et al., 2023).
The Research - Development Institute for Grasslands Brașov (ICDP Brașov), which coordinates the project "*ADER 15.3.2 - Research on the development and promotion of mechanization technologies for the reconstruction, maintenance of permanent grasslands, environmental protection, and biodiversity conservation using new specialized equipment*", has carried out, within the stage "*Identifying solutions to reduce or eliminate degradation factors in locations with a high degree of zonal extrapolation*", an experimental field test in collaboration with the National Institute of Research - Development for Machines and Installations for Agriculture and Food Industry (INMA Bucharest).
This test involved a prototype grassland planer in aggregate with fertilization equipment (during the autumn season), aiming to optimize the work process and surpass the performance of existing technologies.

MATERIALS AND METHODS

At ICDP Brașov, a grassland maintenance technology has been developed, consisting of operations such as clearing mounds and low-value vegetation, fertilization during the years of exploitation, and cleaning residual plant debris left after grazing (Mocanu et al., 2024).
During experiments aimed at determining energy efficiency indices under operational conditions, a New Holland TL 100A tractor - an existing piece of equipment at ICDP Brașov - was used, coupled with the grassland planer experimental model in aggregate with the fertilization equipment for field testing (Figure 1).



Figure 1. Grassland planer experimental model in aggregate with the fertilization equipment

The experimental model is a modular agricultural implement consisting of a fertilization unit mounted directly onto a grassland clearing planer.
The fertilization equipment comprises the following main components: assembled frame, fertilizer boxes, mechanism for transmitting movement to the dosing devices and agitator drive shaft.
The grassland clearing planer consists of the following main subassemblies: frame with a three-point hitch for tractor attachment and working units composed of two U-profile skid rails, connected by two transverse profiles, two rows of adjustable vertical chisel-type knives for varying cutting depth and two rows of inclined horizontal knives, mounted behind the chisel knives, positioned in two different planes with opposing inclinations.
During the operation of the aggregate, consisting of the fertilization equipment mounted on the grassland planer, the pivoting drive wheel, which is kept in contact with the ground by an adjustable spring, transmits rotational motion through a chain transmission, two equal bevel gear transmissions, and a coupling bushing to the Norton-type gearbox. This gearbox allows for multiple speed selections of the main shaft through a stepped gear set, enabling the adjustment of the desired

fertilizer application rate and transmitting motion to the distributors, ensuring precise dosing and uniform spreading of the fertilizers across the field surface.
Subsequently, the grassland planer begins its operation, with the vertical chisel-type knives fragmenting mounds, uneven surfaces, and manure in a vertical-longitudinal plane, while the inclined horizontal knives cut and evenly spread the fragments over the soil. This process creates optimal conditions for their assimilation by plant roots.
The pivoting drive wheel allows for motion transmission even when the implement is moving along a curve, with a steering angle (tilt) of up to 35° to the left or right. This angle exceeds the turning limit of the tractor's steering wheels, enabling the use of the equipment even during turning maneuvers or on narrow paths, a situation frequently encountered in grassland maintenance operations.
Table 1 presents the main technical characteristics of the grassland planer experimental model coupled with the fertilization equipment.

Table 1 Main technical characteristics of the equipment used in the experiment

Characteristic	UM	Value
Working width	m	3.75
Number of fertilizer boxes	pcs	3
Distance between distribution boxes	mm	125
Fertilizer box volume	dm^3	325
Total weight	kg	1290

The tests were conducted on the experimental fields of ICDP Brașov, following a specific testing procedure.
The average climatic data recorded at the experimental research location were as follows: in August 2024 the average daily temperature was 13°C, with an average precipitation of 74 mm; in September 2024 the average daily temperature was 9°C, with an average precipitation of 56 mm.
The additional equipment used to determine the soil characteristics of the experimental field included: FIELDSCOUT SC 900 digital electronic penetrometer with cone and Extech MO750 portable soil moisture meter.
For weighing to determine the front axle load (to verify the longitudinal stability condition of the experimental model coupled with the wheeled agricultural tractor), a Dini Argeo WWSRF portable platform scale was used. This is a mobile scale with platforms connected via a radio signal (Figure 2).



Figure 2. Aspects of the weighing process for the grassland planer experimental model coupled with the fertilization equipment

The percentage value of the front axle load relative to the total mass of the tractor (Mateescu et al., 2015) TL 100A New Holland type, when coupled with the experimental model, is given by equation (1):

$$\frac{M_{Tpf}}{M_{Tpt}} \cdot 100, [\%] \quad (1)$$

where: M_{Tpf} is the mass of the tractor on the front axle (kg) and M_{Tpt} is the total mass of the tractor (kg).
According to internal vehicle circulation regulations, the mass on the front axle must not be less than 20% of the tractor's own weight.
The traction force was determined using the tensometric method with a computer running the nSOFT software package. The following materials and equipment were used:
- 120 Ω strain gauges, type KFG-6-120-C1-11 N15C2, mounted on the tractor's tie rods (Figure 3a);
- 220V AC power supply (Figure 3b);
- Laptop with QuantumX 1615 data acquisition system, featuring 2 modules and 32 measurement channels (Figure 3c).

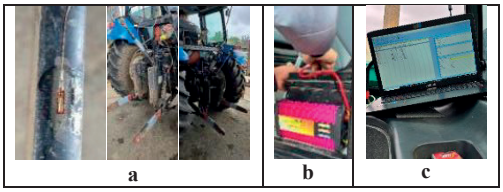


Figure 3. Materials and equipment used for determining traction resistance force

The traction power P_{tr} was calculated based on the travel speed v_l and the traction force F_{tr} determined earlier, using the following equation (2):

$$P_{tr} = \frac{F_{tr} \times v_l}{270}, \text{ HP} \quad (2)$$

where F_{tr} is measured in N and v_l in km/h (Marin et al., 2012).

RESULTS AND DISCUSSIONS

The experimental plot belonging to ICDP Brașov can be visualized as a raster-type map from the Google Earth archive (Figure 4).

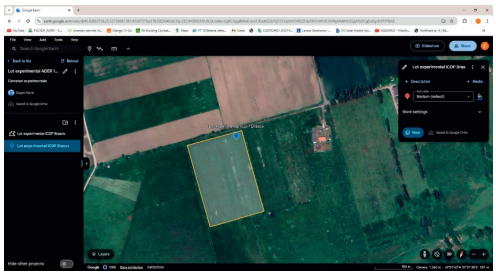


Figure 4. View of the experimental plot on a raster map – Google Earth

On-site measurements conducted on the experimental field plots at ICDP Brașov included the determination of the following physical soil properties: soil penetration resistance and soil moisture content.

Soil penetration resistance is a significant indicator for characterizing the physical-mechanical properties of the soil and its consistency. It correlates with soil plowing resistance (Jiang et al., 2020). The measurement of soil penetration resistance was carried out using the FieldScout SC 900 digital cone penetrometer, where the penetration force was manually applied by the operator. The measurement process included the following steps:

- selection of the cone type: 1 cm² and 60° tip angle;
- attachment of the rod to the cone by screwing them together and mounting them to the "data logger";
- saving the data into the "data logger" by pressing the ON button.

The penetration resistance measurements were carried out at various depths and multiple points (Table 2) to ensure an accurate evaluation. High penetration resistance values (>2000 kPa) indicated strong compaction, which may lead to reduced soil permeability and hinder root development.

Table 2. Average values of soil penetration resistance

No.	Soil depth (cm)	R1	R2	R3	Average soil penetration resistance (kPa)
1	2.5	170	171	179	173
2	5	1370	1362	1376	1369
3	7.5	2705	2701	2705	2704
4	10	3890	3897	3904	3897
5	12.5	3685	3682	3692	3686
6	15	4215	4215	4211	4214

The water content expressed as a percentage of soil volume was measured directly using the Extech MO750 portable soil moisture meter (Hilal et al., 2022).

Moisture values were measured at an average soil temperature of 24°C.

The results obtained from the experimental field measurements for average soil moisture values are presented in Table 3.

Table 3 Average values of soil moisture content

No	Soil depth (cm)	R1	R2	R3	Average soil moisture (%)
1	0-10	8.8	13.1	8.2	**10.03**
2	10-20	8.6	12.8	8	**9.80**
3	20-30	9	13.8	9.1	**10.63**

Figure 5 presents field aspects during the measurement of soil penetration resistance using the FieldScout SC 900 digital cone penetrometer, as well as the determination of soil moisture content expressed as a percentage of soil volume using the Extech MO750 portable soil moisture meter.



Figure 5. Field measurements of the main physical soil properties: penetration resistance and soil moisture content

Analyzing the data from Table 3, it can be observed that the average soil moisture content falls within the optimal range for the application of solid mineral fertilizers.

The traction power was calculated based on the working speed (v_l) of the implement and the traction force (F_{tr}) for two gear levels (V_1 and V_2) of the tractor in the aggregate, both ascending and descending a slope. The results are presented in Table 4.

Table 4. Measured working speed, traction force, and calculated traction power for the experimental model

	Average working speed		Average traction force	Average traction power	
	ascending the slope		ascending the slope	ascending the slope	
	[m/s]	[km/h]	[daN]	[CP]	[kW]
V_1	1.8	6.48	811.7	19.48	14.32
V_2	1.89	6.82	871.3	22.01	16.18
	descending the slope		descending the slope	descending the slope	
V_1	1.82	6.55	801.2	19.44	14.29
V_2	1.94	6.98	852.8	22.05	16.21

Figure 6 presents a graphical representation of the variation of the determined indices at two speed levels for the experimental model coupled with the TL 100A New Holland tractor while ascending the slope.

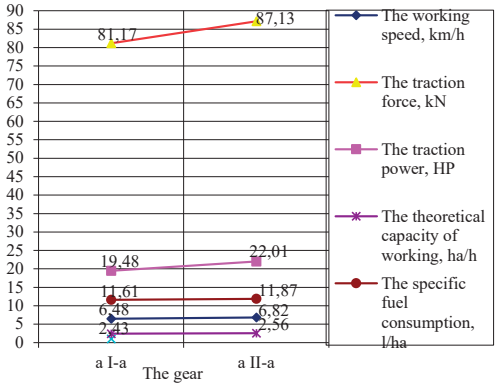


Figure 6. Graphical representation of the variation of determined indices at ascending the slope

Figure 7 presents a graphical representation of the variation of the determined indices at two speed levels for the experimental model coupled with the TL 100A New Holland tractor while descending the slope.

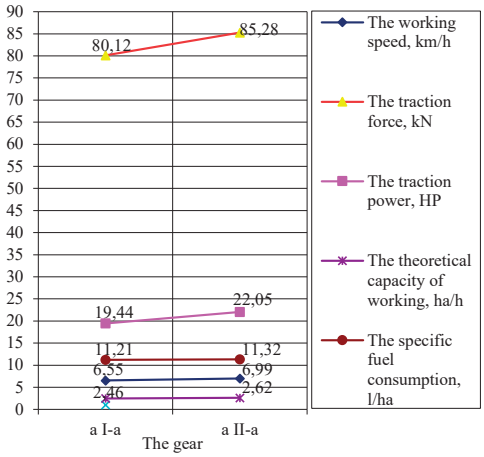


Figure 7. Graphical representation of the variation of determined indices at descending the slope

Table 5 presents the results of technological parameters during operation in aggregate with the TL 100A New Holland tractor on the ICDP Brașov experimental plot, for grassland fertilization over an area of 2 ha, in stubble land.

Table 5. Technological parameters during working in aggregate with the TL 100A New Holland tractor

Specification	UM	Values
Fertilizer type	-	NPK
Average displacement speed	km/h	2.56
Surface	ha	1
Real working time, T_1	min	24.7
Working hourly capacity at real time, W_{ef}	ha/h	2.43
Capacity of working per hour at shift time W_{07}	ha/h	1.80
Technological safety coefficient, K_{41}	-	0.98
Technological safety coefficient, K_{42}	-	1.0
Reliability coefficient, K_4	-	0.98
Shift time utilization coefficient, K_{07}	-	0.83
Fuel consumption per ha	l/ha	10.82

During the operational experiments conducted over an area of 1 ha, the grassland planer experimental model coupled with the fertilization equipment performed efficiently throughout the entire period. Under these conditions, it achieved: an operational safety coefficient of 0.98, a performance working capacity of 1.80 ha/h and a fuel consumption rate of 10.82 l/ha.

Figure 8 presents an aspect from the operational experiments conducted on the experimental plot near ICDP Brașov.



Figure 8. Aspect from the operational experiments

CONCLUSIONS

From an operational perspective, the technical equipment achieved technological parameters that comply with agrotechnical requirements for the application of solid chemical fertilizers.
During the tests, the experimental model coupled with the TL 100A New Holland tractor demonstrated good performance, achieving an average working capacity of 2.43 ha/h and an average fuel consumption of 10.82 l/ha.
The experimental research enabled the validation of the technical and technological solutions implemented in the design of the experimental model.

ACKNOWLEDGEMENTS

The results presented in this article were obtained with the support of MADR through the "Sectoral Plan - ADER 2026", financing contract no. 15.3.2 / 17.07.2023, ADER project 15.3.2. Research on the development and promotion of mechanization technologies for the reconstruction, maintenance of permanent grasslands, environmental protection and biodiversity conservation using new specialized equipment, managed by the Research - Development Institute for Grasslands Brasov - Mechanization Laboratory.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

METHODS FOR DETERMINING PROTEINS AND AMINO ACIDS AND THE IMPORTANCE OF THEIR USE FOR IDENTIFYING SUSTAINABLE SOURCES OF DIETARY PROTEINS. A REVIEW

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Abstract

The aimed of this review is to highlight the importance of sustainable vegetarian protein sources in a world with a growing population in which dietary principles have also changed. This involves using sustainable non-animal protein sources. Nevertheless, the quantification of the nutritional quality of food sources also involves the normalization of the essential amino acid content with the protein content. For this, new methods for the determination and analysis of proteins individually or simultaneously with amino acids are needed, with possible advantages such as being simple, inexpensive, fast and accurate, etc. In this review, we try to summarize the benefits of a sustainable vegetarian diet (rich source of proteins), but also the importance of the continued development of simpler and faster methods to determine protein quality and content. The use of spectrophotometric methods for the measurement and analysis of proteins is of major interest, as they are generally simple, fast and with high sensitivity, as well as the adaptation of traditional methods such as the traditional biuret reagent.

Key words*: protein, amino acids, sustainable sources, vegetarian diet.*

INTRODUCTION

Our food systems play a major role in global greenhouse gas (GHG) emissions, making them a key target for addressing the climate crisis (Poore & Nemecek, 2018). Given the many elements of a sustainable food system (e.g., water, biodiversity, energy use, and food security), a holistic approach is clearly needed to achieve sustainability (Béné et al., 2019). Knowing that food system represents 20 to 30% of the global GHG emissions (Chai et al., 2019), we may understand that promoting sustainable foods that have minimal environmental impact, we can enhance health and contribute to the well-being of local communities. According to the official United Nations website, these foods are grown, produced, and distributed in ways that protect natural resources, prevent pollution, and reduce greenhouse gas emissions.

Although the technological innovations in agricultural sector, food availability and security have increased significantly (Kalmpourtzidou et al., 2022), diets with low vegetable, fruit, wholegrain and high in red and processed meat intake are dominant worldwide (Springmann et al., 2018; Kalmpourtzidou et al., 2020).

Sustainable food features include local sourcing, minimized food waste, organic farming methods, and low water and energy usage. Shifting towards plant-rich diets is expected to deliver benefits for human health, the environment, and animal welfare (Allenden et al., 2022).

Proteins are the building blocks of life. The basic structure of protein is a chain of amino acids - protein foods (plant and animal sources) are broken down into parts called amino acids during digestion. A balanced diet, rich in carbohydrates, proteins, and lipids, is crucial for supporting physiological functions and maintaining good health. Among these, proteins are particularly important as they are essential macronutrients for cell growth and repair in the body. The demand for protein sources has been rising, especially among

athletes, who seek to increase muscle mass and prevent muscle breakdown. While animal proteins are highly digestible, they may not always be accessible or widely consumed due to factors like high cost or lifestyle choices (such as vegetarianism and veganism). As a result, scientists and the food industry are actively exploring alternative protein sources, including plant-based and fungal proteins.

It is known that vegan diets might reduce the risk of premature mortality, NCDs, and excessive weight gain (Moreno et al., 2021). However, it is important to keep in mind that the health benefits of any diet partly depend on the variety of foods consumed and the way in which food is stored, processed, and prepared (Comerford et al., 2021). Complete proteins, typically found in animal-based sources, are highly bioavailable, but also contribute to environmental harm and an increased risk of diet-related chronic diseases. In recent years there has been growing awareness about the health benefits of replacing animal-based proteins with plant-based alternatives, particularly in developed countries. Higher protein quality per meal in the vegan scenario is possible when smart combinations are made by also replacing other meal components and real-life practice shows that people usually replace an animal-based product with a similar plant-based alternative, without making other changes within the same meal (Gehring et al., 2021). Key challenges to improve the consumer acceptability and market value of plant-based proteins would be in achieving nutrient equivalency and enhance bioavailability and digestibility while maintaining the same physicochemical properties, taste, texture, as animal proteins (Ulhas et al., 2023).

Standardized analytical methods are essential for accurately determining the protein and amino acids content in food. Various methods, such as Kjeldahl, Lowry, Bradford, and total amino acid content analysis, are employed across different food industries to quantify protein levels. Accurate measurement of protein content is crucial, as it often influences the economic value of food products and can affect the financial viability of emerging industries focused on alternative protein production.

MATERIALS AND METHODS

In order to achieve our goals, we used the method of documentation on site and on line, both letric and electronic sources, and start to identifying, accessing and selecting of several significant scientific results from the area of our proposed topic, published in different journals indexed in WOS, Clarivate Analytics, Scopus and Springer databases.

The most valuable were highlighted in the present review and were also cited and included in the references chapter.

RESULTS AND DISCUSSIONS

Methods for protein and amino acids determination

The methods used for quantitative protein determination can vary significantly in terms of relative response factors, matrix dependency, sensitivity, sample preparation effort, instrumentation, cost, duration, and sample size (ranging from picogram to kilogram) (Reinmuth-Selzle et al., 2022).

Spectrophotometric methods for protein determination and analysis are highly valued due to their simplicity, speed, and high sensitivity. These protein assays have many uses in the food, plant breeding, pharmaceutical and biomedical industries, as well as in forensic science and a variety of other research fields (Mihalcea et al., 2024). However, the measurement of the nutritive quality of food samples also requires the nominalization of essential amino acid content to that of protein (Mihalcea et al., 2024).

The most commonly used methods for measuring protein content in foods include the Kjeldahl method, Dumas method, and direct measurement techniques, like UV-spectroscopy and refractive index measurement, each have their own set of advantages and disadvantages. ***The Kjeldahl method*** is a reference technique for protein quantification in food, but it is time-consuming and requires large protein amounts (typically 5 g), making it less suitable for small sample sizes (Wang et al., 2016). The Kjeldahl method involves the digestion of food with a strong acid so that nitrogen is released, which is then quantified using a titration technique (Hayes, 2020). Protein quantity is calculated

from the nitrogen concentration of the food using a conversion factor (usually 6.25, equivalent to 0.16 g nitrogen per gram of protein), which is the standard method for protein measurement but has its disadvantages (Hayes, 2020). As discussed previously by Maehre et al. (2018) and Harrysson et al. (2018), it does not measure true protein and the conversion/correction factor of 6.25 is not suitable for all protein types and should be corrected based on the amino acid composition of the protein in question. Several studies have determined species-specific nitrogen correction/conversion factors. For instance, a conversion factor of 5.6 is recommended for shrimp and fish, 5.4 for cereal products, and 4.59 for red seaweed. However, other researchers have suggested conversion factors of 4.9 for fish and 4.7 for flour (Maehre et al., 2018).

The Dumas method is fast and chemical-free but costly to set up and lacks accuracy as it does not measure true protein, while UV spectrophotometric methods such as the Biuret, Bradford, and Lowry methods are easy to use, inexpensive, and capable of quantifying small amounts of protein (Hayes, 2020). However, they can give false positive protein readings depending on the sample preparation method used and solubility of the test sample; while direct amino acid analysis involves hydrolysis of the protein with HCl and subsequent quantification of the amino acids using HPLC, but unfortunately is required initial investment in HPLC equipment, hydrolysis step and is time consuming but is the most accurate method (Hayes, 2020).

Protein content is a critical factor in the technological performance of products. Currently, the ISO standard method for determining protein content is the Kjeldahl method, which is time-consuming and requires specialized instrumentation. For this reason, the use of rapid methods to quantify protein would greatly facilitate the monitoring (Hueso et al., 2022). Methods as Bradford assay, Dumas method and the bicinchoninic acid assay (BCA) have been taken into consideration. For a milk sample, the protein content obtained from Bradford assay and Dumas method was consistent with Kjeldahl values (Hueso et al., 2022). In contrast, BCA protein levels were significantly different when compared to Kjeldahl and no method was proved to be suitable for protein determination in permeate samples (Hueso et al., 2022). In comparison with the official method, the Bradford assay provided the best quantitative results and is recommended for a quick, economical, and easy determination of total protein content in milk and retentate samples, as its simple protocol requires neither expensive equipment nor experienced analysts for data acquisition (Hueso et al., 2022).

Another example of an improved method is the modification of the traditional biuret reagent by replacing copper sulfate and Seignette salt with an alkaline alcohol solution and insoluble copper phosphate, which requires only about 50 mg of biological material to quantify three different components in each sample (Mihalcea et al., 2024). The method offers several advantages, including simplicity, low cost, speed, and accuracy. It involves weighing a single sample to determine both protein content and essential amino acids, lysine and tryptophan in the same biuret supernatant. It is important that new methods for the simultaneous determination of protein and two of the most important amino acids, lysine and tryptophan, are emerging.

Amino acids (AAs) are essential nutrients for life, playing a crucial role in maintaining vital body functions, including protein synthesis, healthy growth, tissue repair, and balanced metabolism. The most of AAs, except tryptophan, phenylalanine and tyrosine, lack a strong chromophore (Yuliarti et al., 2021), such as no ultraviolet absorption and no fluorescence. For example, the amino acids composition in the samples of some herbal raw materials was studied by ***gas chromatography-mass spectrometry (GC-MS) method*** (Savych et al., 2021). Good results were obtained analysing the amino acids composition of *Centaurium erythraea* Rafn. and *Gentiana cruciata* L. by HPLC method with a pre-column derivatization 9-fluorenylmethyl chloroformate (FMOC) and o-phthalaldehyde (OPA) (Budniak et al., 2021). ***A new hydrophilic interaction chromatography (HILIC) method*** for free amino acid analysis in honey samples was developed, involving pre-column derivatization with dansyl chloride

(Dns-Cl), sample pretreatment with modified QuEChERS extraction, and liquid chromatographic analysis with UV detection (Kırkan & Aydoğan, 2021). Therefore, AAs are always determined by derivatization to increase detection sensitivity, but there are some drawbacks, such as complex derivatization procedure, incomplete derivatization, derivative instability, reagent interference, expensive reagents and long analysis time (Wada et al., 2021; Figueroa et al., 2023). Therefore, a quick, simple and accurate analytical method for amino acid is needed (Chen et al., 2021). ***A direct method for determining amino acids in plants using HILIC and HPLC-MS/MS*** was established, with sample extraction completed in 10 minutes, making it simpler, faster, and more environmentally friendly, while the determination of 21 underivatized amino acids was completed in 15 minutes with satisfactory recovery, precision, and stability (Chen et al., 2021).

Thin-layer chromatography (TLC) is classically used for routine separations, identification of the individual amino acids or vitamins, and their quantitative determinations. With the advent of the automated multiple development (AMD) technique all steps from application to mixing solvents, development, and drying can be automated. Although such automation is obviously expensive, it is a prerequisite for the wider use of HPTLC in the pharmaceutical industry (Mohammad et al., 2012). High performance thin layer chromatography (HPTLC) involving the use of smaller average particle size of adsorbent in the preparation of TLC plates provides faster separations with reduced zone diffusion and enhanced sensitivity (Mohammad et al., 2012).

An analytical method for the direct determination of amino acids (methionine, proline, glycine, histidine, arginine, and lysine) in biologics was developed using hydrophilic interaction HPLC with UV detection, offering the advantages of simplified sample preparation by eliminating the derivatization step and enabling simultaneous selective quantitative determination of multiple amino acids (Runova et al., 2021).

This ultrasound-assisted acid hydrolysis procedure using HPLC-UV offers a reliable and efficient alternative for AA analysis in plant-based proteins, offering significant cost and time savings (Custodio-Mendoza et al., 2024).

Kowalska et al. (2022) studied and optimized the simple extraction of flours samples followed by free amino acids determination procedures; the conditions of amino acids derivatisation reaction with ninhydrin for chromatographic determination of free amino acids sum.

The drawbacks of HPLC methods are the usage of high volumes of toxic solvents and sample derivatization (Pukleš et al., 2024). Listed drawbacks led to a trend where such products are often analyzed by analytical methods developed by the industries themselves, even though they have not been standardized or validated (Gilani et al., 2008). ***Capillary electrophoresis (CE)*** is often preferred over HPLC as a faster and more environmentally friendly analytical technique, commonly used for analyzing pharmaceuticals and biopharmaceuticals. (Costa et al., 2019; Piestansky et al., 2021).

Microchip electrophoresis (ME) devices have proven to be powerful tools for analyzing amino acids (AAs), offering advantages such as low cost, rapid analysis, minimal sample and reagent consumption, and effective separation, establishing their importance in analytical science (Pukleš et al., 2024). Compared to CE, the method optimization is more demanding due to the short separation path (Tüma et al., 2007).

Sustainable sources of dietary protein

In the European Union, food proteins are predominantly animal-based, primarily from meat and dairy products; almost all soy but also a larger part of pulses and cereals consumed in the European Union are used for animal nutrition (Detzela et al., 2021). The global population is expected to surpass 9 billion by 2050, driving a substantial rise in the demand for food, particularly protein-rich foods and animal feed (Godfray et al., 2010). The rising awareness about the relevance of high-quality proteins in the diet has encouraged the researchers and nutritionists to search for eco-friendly and sustainable protein sources (Kumar et al., 2021).

Innovation and interest in both novel protein sources such as plant, algae, and mycoprotein, and their respective processing technologies have been rising steeply (Rolland et al., 2020).

Some common alternative protein sources for vegan diets include legumes (lentils, chickpeas, peas, and beans), grains (quinoa, barley, and bulgur wheat), nuts and seeds (almonds, chia seeds, and pumpkin seeds), mycoprotein (single cell protein from fungus) and algal proteins (Kaur et al., 2021; Zeng et al., 2022; Qin et al.,2022).

Animal based proteins such as dairy, meat, poultry, and eggs are considered complete proteins because they contain all of the essential amino acids in sufficient amounts (Ulhas et al., 2023). Alternative proteins, on the other hand, are often considered incomplete proteins because they may lack one or more of the essential amino acids (Gorissen et al., 2018). For example, legumes such as beans, lentils, and chickpeas are high in lysine, while grains such as rice and quinoa are high in methionine and cysteine (Hertzler et al., 2020). Most of cereal species have low protein quality due to their small amounts of lysine and tryptophan (Babu & Prasanna, 2014). Therefore, by combining these different plant-based proteins, an individual can obtain a complete protein source that provides all of the essential amino acids needed for optimal health (Gorissen et al., 2018). For consumers not interested in vegan or vegetarian alternatives, meat hybrids, which replace a fraction of meat (e.g., 20% to 50%) with plant-based proteins, could offer a low-threshold option for more sustainable food consumption (Profeta et al., 2021).

Plant-based protein sources including legumes, seeds and nuts, and by-products/waste from food production have been studied and utilized to simulate meat and other analogues (Tachie et al., 2023). Some seeds such as pumpkin, sunflower and hemp have also been used to produce alternative eggs, cheese and flour products respectively (Watson, 2021). Meat, egg, dairy products, bakery products (snacks), beverages, and dietary supplements have been developed from plant and alternative protein sources (Bunge, 2022).

Due to the fact that demand for sustainable sources of dietary protein is steadily growing, providing dietary protein to a growing world population with rising living standards requires the development of sustainable protein sources other than animal meat (Yuliarti et al., 2021). Sesame meal holds significant potential as a source of plant-based protein. Because of its unique amino acid composition, high in methionine and cysteine, and low in lysine, means that consuming it together with soybean protein provides a more complete profile of essential amino acids (S´a et al., 2022). Sesame meal is a by-product rich in protein, produced during the industrial extraction of sesame oil which is currently treated as waste. That it could help meet this need if high quality protein could be extracted from the meal in an environmentally friendly way; the usually method of traditional alkali-soluble acid precipitation extraction process produces proteins with poor functionality and harmful to the environment (Cao et al., 2023).

Hempseed protein is another nutritious and sustainable alternative, offering a rich source of amino acids. It has gained increasing attention for its sustainability and nourishment; therefore, hempseed protein is an excellent potential protein ingredient to be applied to food systems to add nutritional value and promote environmental sustainability (Dong et al., 2024). From a nutritional standpoint, hempseed protein isolate encompasses a complete profile of all the essential amino acids required by adults according to FAO/WHO standards and presents potential bioactive peptides after digestion (Mamone et al., 2019).

Microalgae and macroalgae (Seaweed) are traditional food sources in many cultures, some algal species can have comparable or even higher protein content compared to traditional meat, milk, egg, or plant protein sources (Boukid et al., 2022; Fleurence et al., 2018). Laver (*Porphyra* spp.) is one of the most commonly cultivated seaweeds, typically consumed in its dried form. Seaweed is not only nutritionally excellent, as it is rich in protein, dietary fibre, and vitamin B, but is also used as a raw material for various foods such as sushi and gimbap (Wada et al., 2021). Red seaweeds, including laver, are known for their wide-ranging protein content, varying from 2.7 % to 47 % (Figueroa et al., 2023). The protein content is the most important quality

indicator of laver. Laver's high protein content not only provides nutritional value, but the amino acids such as taurine, alanine, and glutamic acid derived from this protein are also the basis for its distinctive flavour (Jeong et al., 2023). Additionally, dried laver is produced by grinding raw laver and then drying it, which results in minimal visual differences based on its composition and gives it a characteristic black colour (Kim et al., 2024). Consequently, it is challenging to determine the protein content of dried laver without specific quantitative methods such as Kjeldahl Method (Kim et al., 2024).

Legumes have been the world's second most important source of staple food after cereals as "poor man's meat". To date, several legumes have been studied and proposed as protein alternatives for human consumption and among them: soybean is the most prominent in terms of economics (Cheng et al., 2019). Knowing that soy protein is rich in all the nine amino acids that are necessary for human nutrition (Kudełka et al., 2021), plant-based meat alternatives have commonly been based on products derived from soy due to its high protein content (Cober et al., 2022). Heaving a higher protein level than both cereals and potatoes, they are filled with all the important amino acids that the human body needs (Guo et al., 2023). It can be utilized as a replacement for animal proteins in the food sector since it has nutritional and biological benefits similar to those of animal proteins (Huang et al., 2019). However, natural soy protein isolate contains a large number of hydrophobic groups, which seriously affects the solubility and emulsification of soy protein, resulting in its functional properties not being able to be well developed, limiting the application of soy protein in industry (Arfat & Benjakul, 2013). Phosphorylation of sodium tripolyphosphate reduces protein molecule size and increases intermolecular interactions, making it a dispersing additive in food processing and offering a reference for developing functional properties of industrially modified soy isolate proteins in the food industry (Guo et al., 2023).

Wheat gluten, also known as 'seitan,' is used as a binding agent combined with soy to create plant-based meat. The unique viscoelastic properties of wheat gluten allow it to form a resilient gel making it useful in the preparation of thickeners, fortifiers, and texturing agents (Pietsch et al., 2019). Chickpea is also a rich source of dietary protein (17-22%), has low allergenicity, high solubility and bio-functionality making it an interesting candidate as an alternative ingredient for plant-based meat (Boukid et al., 2021). In addition to chickpea, pea protein has also drawn interest from researchers as a potential additive in alternative meat products (Lu et al., 2020).

Extensive research has been conducted on several underutilized legumes with similar nutritional properties to soybean; one of these legumes is velvet beans (Baby et al., 2023). Though it is rich in protein (27%), complementary amino acid pattern to that of cereal grains exerts health beneficial properties, including antiparkinsonian, antidiabetic, and anticancer properties, it is limited for human consumption due to the presence of antinutrients (Baby et al., 2023).

Pulses (beans, peas, lentils, cowpeas, chickpeas, pigeon peas, etc.) being the second most important food source for humans, following cereals, play a key role in human nutrition, being rich in proteins, complex carbohydrates, essential vitamins, minerals, and phytochemicals, while being low in fats. Pulses are also considered the most suitable for preparing protein ingredients (Concentrates and isolates) because of their high protein content, wide acceptability and low cost.

Protein content of different pulses varies with genotypes, germination, environmental conditions and application of fertilizers during growth and development (Singh, 2017; Isticioaia et al., 2020). The pulse proteins have essential amino acids composition complementary to cereals and are naturally gluten-free, thus, safe for the patients suffering from gluten intolerance/allergies (Shevkani et al., 2019).

Applications in which pulses are used includes bakery and pasta (because of the essential amino acid content complimentary to cereals makes them particularly suitable for improving the protein quality of cereal-based foods (Shevkani et al., 2019), imitation milk and bean curds (preparation of non-dairy/imitation milks, beverages and bean curds), meet products (the effect of incorporation of lower levels of (1.5,

2.5 and 5%) chickpea protein concentrates on the properties of cooked sausage and reported that chickpea proteins not only reduced cooking yield and lipid oxidation but also increased color stability, total antioxidant capacities, protein content and acceptability by consumers (Ghribi et al., 2018), encapsulation material and edible/biodegradable films.

The seeds of flowering plants typically store significant amounts of seed protein, including storage proteins and lectins. The amount of protein found in seed varies from 10% (in cereals) to 40% (in certain legumes and oilseeds) of the seed dry weight, which forms a major source of dietary protein (Liu et al., 2017). The 16 proteins identified in faba bean were mainly composed of glutamic, arginine, lysine, glycine, leucine, cysteine, and methionine, which are essential to human life (Liu et al., 2017). The flours are essential and the most commonly used raw material for bakery products, however, due to the properties of proteins in gluten-free flours, their usage is limited (Kowalska et al., 2022). On the other hand, the nutritional properties of gluten-free flours are their main advantage due to the well-balanced composition of the amino acids with high biological value (Ciesarová et al., 2021).

Another rich source is found in fresh and preserved mushrooms which have been appreciated by humans since ancient times for their organoleptic, nutritional and medicinal properties (Wani et al., 2010). Although they are low in calories, mushrooms are a good source of high-quality protein, amino acids, dietary fibre, vitamins (vitamin B2, folate and niacin), as well as many healthy minerals (iron, calcium, phosphorus, potassium, selenium, zinc and copper) and phenolic compounds (Mattila et al., 2001).

Although not a source of plant protein, fish by-products, such as frames, trimmings, and viscera from commercial fish species, are rich in proteins and could serve as an economical source of bioactive peptides and functional protein hydrolysates for the food and nutraceutical industries (Nikoo et al., 2023). Despite this necessity, within the seafood sector, the increased use by-products is happening relatively slowly because the seafood industry focuses on its primary raw materials and products that require minimal processing (Wassef et al., 2023).

Sustainable diets and environmental impact

Sustainable diets focus on enhancing public health, food security, and reducing the environmental impact of the food system by promoting a plant-based diet and reducing the consumption of animal products (Lonnie & Johnstone, 2020). Meat and dairy, the main protein sources in an omnivorous diet, contribute the most to the food-related ecological footprint (Borkent et al., 2024). The food systems underlying these diets are responsible for a third of global greenhouse gas emissions (Crippa et al., 2021) and influence several other environmental indicators (Willett et al., 2019). A transition to a more sustainable diet-one that contains more plant-based products and less meat, could increase food availability while releasing the burden on the environment (Chen et al., 2019).

Studies suggest that the environmental impact of food production is higher for omnivorous diets and lower for vegetarian/vegan diets (Moreno et al., 2021). Fortunately, the alternative protein market is rapidly growing as consumers are becoming more conscious of the impact of their food choices (Kaur et al., 2021). The effects of food production on climate change have led to decreased agricultural yields and increased food insecurity in some regions of the world (Rockstrom et al., 2009). This, combined with the rapidly growing global population, makes current food systems unsustainable. According to the WRI, a combination of changes will be required to reduce the impact of our diets on the environment, including a shift in our food choices, changes in food production (sourcing and transformation), and reductions in food losses and waste (World Resources Institute, 2018).

Although the environmental impact of animal-sourced foods can be reduced by reducing our consumption of red meat, further benefits can be gained from changes in farming practices, such as recycling manure as fertilizer for crops, converting manure into biogas, and/or feeding cattle on grasslands instead of (or alongside) animal feeds (Moreno et al., 2021).

In terms of plant-sourced foods, agricultural practices increasingly rely on the use of chemical fertilizers to restore nutrients to the soil, thereby enabling farmers to plant just 1 crop, without the need for rotational cropping (Lacour et al., 2018; Partal et al., 2020, 2023).
The urgent need to shift to plant-rich diets is now considered a vital strategy in achieving a global sustainable food system and in combating an important driver of climate change (Allenden et al., 2022). Both collective and individual level, food behaviours and food choices represent major levers of action against the ongoing environmental disaster (Rabès et al., 2020).

CONCLUSIONS

The development of new methods for the determination of protein and amino acids is essential for the advancement of research and the food industry, with multiple positive implications, such as: increased accuracy and reliability (more accurate determination of protein and amino acid content, essential for assessing the nutritional quality of food products), fast and efficient (modern methods, such as spectrophotometric and machine learning techniques, provide fast and efficient results, saving time and resources in laboratories and industries), wide applicability (these new methods are versatile and can be applied to a wide range of food products), promoting healthy diets (the correct determination of proteins and amino acids is essential for creating food products with adequate nutrient content) and innovation and sustainability (the development of these methods can support research into alternative protein sources, such as plant protein or protein from sustainable sources, contributing to innovative solutions to global food challenges).
The use of protein from sustainable sources is not only a necessity to protect the environment and ensure a balanced diet, but also an opportunity to encourage more responsible and affordable agriculture.
In conclusion, progress in the development of protein and amino acid determination methods is essential to improve food quality, production process efficiency and nutritional sustainability on a global scale.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

PARTICULARITIES REGARDING THE TENDING OPERATIONS IN FOREST SHELTERBELTS

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Abstract

In order to optimize the ecological and economic functions that forest shelterbelts perform, it is necessary to carry out appropriate maintenance work, through which certain trees are extracted and their density is reduced. In this regard, tending operations were carried out in forest shelterbelts with different compositions and ages to ensure sufficient and as uniform space as possible for the growth of the remaining trees. This spacing of the trees has an influence on the growth and quality of the trees but also on the resistance to disruptive climatic factors and implicitly on the protective functions they perform. The gradual and periodic reduction of the number of trees corresponding to the age and stage of development of the shelterbelts is carried out according to the principles adopted in forestry, mainly applying negative selective thinning, namely the extraction of small, poorly formed, injured, diseased, and dry trees. However, unlike stands, care will be taken to ensure that the extractions carried out do not affect the semi-penetrability of the forest shelterbelts, which must perform specific functions. By experimenting with different degrees of thinning, the aim is to establish an optimal density, through which the forest shelterbelts can exercise their protective functions, and the equilibrium state of this agroforestry system is not affected.

Key words*: forest shelterbelts, tending operations, cleaning, thinning, agroforestry system.*

INTRODUCTION

Since forest shelterbelts are the most representative agroforestry systems in our country in terms of importance and occupied area, in the last three decades there have been constant concerns for their establishment in as large areas as possible (Adam et al., 2012; Constandache et al., 2013; Dănescu et al., 2007; Dănescu et al., 2007; Dănescu et al., 2017). During this period, forest shelterbelts for the protection of fields and roads were established by farmers and/or authorities in different areas of the Romanian Plain, to protect agricultural crops and roads from the effects of disruptive climatic factors such as: droughts, strong winds and heavy snowfalls (Dolocan et al., 2022; Nițescu, 2023). Although they occupy small areas compared to what needs to be protected in the Romanian Plain (Costăchescu, 2010, Mihăilă et al., 2022), which is vulnerable from a climatic point of view (Prăvălie et al., 2017), they have a proven favourable effect in terms of improving the local microclimate and implicitly increasing agricultural production and the quality of agricultural production (Vasilescu, 2015). Climatic factors, soil type, species composition and forest shelterbelts management significantly influence the efficiency of these forest formations. Since climatic factors cannot be influenced by humans, and the species composition is established according to the soil type when forest shelterbelts are established, it is important that their management to be done constantly and systematically, from establishment to harvesting and not just until the trees are completely closed. Therefore, maintaining and consolidating the protective and productive role of forest vegetation in forest shelterbelts is achieved by carrying out all silvicultural works. In the context of climate change, these tending operations must be adapted to ensure the resilience of forest stands and to maintain their long-term protective role. Only in this way, forest shelterbelts can fulfill as many ecosystem services as possible, from improving the microclimate, improving soil fertility and conservation conditions, preserving and increasing agricultural production, to

increasing biodiversity, storing carbon and improving the quality of air, water and landscape (Costăchescu et al., 2010; Lupe, 1952; Neșu, 2012). The works will be carried out depending on the condition and stage of development of the forest vegetation in the shelterbelts. Their timely and appropriate implementation influences both the increase in the value and resistance of the forest vegetation to disturbing factors and the full use of the production capacity of the site. Just as the tending operations of stands, correctly conceived and applied, represent an indispensable and efficient means for the sustainable management of cultivated forests (Petrescu, 1971), the same concern must exist for the forest vegetation in the forest shelterbelts. Therefore, the following tending operations will also be carried out here: cleaning, thinning, and hygienic cutting. These works are essential for ensuring an optimal structure and implicitly fulfilling the functions for which the protective forest shelterbelts were created.

The influence that forest vegetation has on crops, as well as the interactions between them, is reflected in the benefits that the newly created system brings, namely ecological, economic and social benefits, some of which predominate depending on the type of forest cover existing in a given area (Costăchescu et al., 2010).

To date, no research has been conducted in our country to substantiate the method of carrying out tending operations in forest shelterbelts and other agroforestry systems, so it was considered necessary and appropriate to introduce these aspects into the research theme of the FORCLIMSOC Nucleu Program (Tudose et al., 2023-2026). "Technical forestry guidelines for the establishment and tending operations of forest vegetation in protective forest shelterbelts" have been developed (Annex to Ord. MAAP No. 636 of 23.12.2002), but they are based only on the experience gained in the tending operations of stands.

In addition, for field protection forest shelterbelts these norms are not mandatory. Due to the costs of these works and the lack of technical personnel to apply them and verify the correctness of their implementation, these guidelines have not been applied. Sporadically, some shelterbelts have been carried out in hygiene works and firewood extraction works.

The present paper aims to analyse the particularities regarding the implementation of tending operations in protective forest shelterbelts, of different ages and compositions, highlighting the thinning methods and the effect of these interventions on forest vegetation, thus contributing to the optimization of management strategies of these essential agroforestry systems in the plain area of our country. Also, practical aspects related to the management of these forest formations under different pedoclimatic conditions and in the context of current climate change are addressed.

MATERIALS AND METHODS

In 2023, three experimental setups were installed in three different forest shelterbelts. Each experimental setup consists of three experimental blocks, each block having four variants. The four variants correspond to a control variant and the three thinning intensities applied, namely: strong, moderate and weak, through which a larger number of exemplars were extracted, corresponding to the strong variant, to minimal tree extractions corresponding to the weak variant. In the selected forest shelterbelts, two tending operations types were carried out, namely: cleaning and first thinning.

In order to establish the most appropriate tending operations for the selected shelterbelts, an analysis of the structural characteristics of the forest vegetation was previously carried out, in the horizontal plane: composition, consistency, density, average diameter and basal area, and in the vertical plane, average height, by species and for the forest shelterbelts as a whole (Mihăilă et al., 2024).

Cleaning works were carried out in two experimental setups: i) the forest shelterbelts formed by Siberian elm (*Ulmus pumila* L.) and honey locust (*Gleditsia triacanthos* L.), established in the spring of 2020 at the Perișoru Farm, Călărași County, which is 5 years old; and ii) the protective forest shelterbelts formed by greyish oak (*Quercus pedunculiflora* K. Koch), flowering ash (*Fraxinus ornus* L.) and wild pear (*Pyrus pyraster*) established in the

fall of 2017 at the Grădiștea Farm, Călărași County, 7 years old.
The first thinning was carried out in one experimental setup, the forest shelterbelts formed by Siberian elm and honey locust, established in the spring of 2018 at the Grădiștea Farm, Călărași County, 7 years old.
The size of the test areas is 140 m^2 (10 x 14 m), at the Perișoru Shelterbelt and in the Grădiștea Shelterbelt, with Siberian elm in the composition and 100 m^2 (10 x 10 m) in the Grădiștea Shelterbelt, with greyish oak and flowering ash in the composition.
To determine the intensity of thinning work in the forest shelterbelts, all the trees in the sample areas were inventoried, both those remaining in the shelterbelts following the tending operations and those extracted, measuring their circumference at 1.30 m from the height of the tree and their total height (H).
Based on the recording of the circumference of the trees, the diameter at breast height (Dbh) of the trees was determined, in centimetres, which was the basis for determining the basal area.
The basal area, BA, which represents the area corresponding to the diameter of the base of a tree, was determined with the formula: $BA = \pi \cdot Dbh^2/4$.
The basal area for each sample area was determined by aggregating the basal area of all trees in the area.
Recording the number of trees, as well as determining the basal area, before and after the thinning work, were the basis for determining the thinning intensities. The thinning intensity by the number of trees is more expeditious, but because the trees were not relatively evenly distributed, it was chosen to establish the intensity of the extraction works by the basal area, which presents greater accuracy in the specific conditions of the analysed forest shelterbelts.
The intensity of the basal area (i_{BA}), expressed as a percentage, was determined as the ratio between the basal area of the extracted trees and the cumulative basal area of the trees in the sample area before the thinning work, according to the formula:
$i_{BA} = BA_e/BA_i \cdot 100$
The recording of tree diameters and heights was also done one year after the tending operations were carried out, to analyse the reaction of the remaining trees as a result of the reduction in the number of trees. In particular, the recording of height is important in forest shelterbelts, because the protected agricultural area depends on the size of this parameter. The data collected have been statistically processed and interpreted, using Microsoft Excel and Statistica 7.1.

RESULTS AND DISCUSSIONS

By carrying out the tending operations, the development of forest shelterbelts is supported and directed to fulfill the protection and production functions in the best conditions and with maximum efficiency. Failure to carry out these works or their omission significantly reduces the beneficial effect of the forest component within these agroforestry systems, leading to the degradation or premature aging of the forest vegetation (Figure 1).

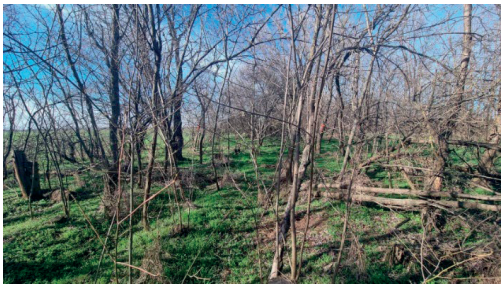


Figure 1. Degraded forest shelterbelts where the tending operations were not carried out

Since only two to three species were used in the afforestation works to create the studied forest shelterbelts, a small number of rows (5 and 7), specific to forest shelterbelts for crop protection, and a planting scheme of 2 x 1 m, after the trees reach crown closure, there was no danger of some specimens being overwhelmed by others, as happens in naturally regenerated stands. For this reason, the maintenance works in these shelterbelts do not include early cleaning works, but only cleaning and thinning works, depending on the age of the forest shelterbelts and the development of the trees within them. These works are necessary to conveniently direct the development of the trees and accelerate their growth.

Four years after planting, when the structure of the forest vegetation was assessed, in the forest shelterbelts with Siberian elm and honey locust from Perișoru, the total number of trees per hectare was 37% lower than at planting, Siberian elm by 39% and honey locust by 35% (Mihăilă et al., 2024). Although the number of trees decreased, maintenance work was still necessary because many specimens, especially Siberian elm, had 2-4 shoots, which competed with each other. There were also areas where the specimens were very dense and, under conditions of competition, their development was not achieved in optimal parameters.

The average diameter in the sample areas varies from 4.2 to 6.1 cm and the height from 4.8 to 5.4 m (Mihaila et al., 2024) so the silvicultural work indicated and necessary in this shelterbelt is clearing. The purpose of these works is to obtain a well-proportioned and spaced mixture so that the remaining trees accelerate their height growth and develop their crown as well as possible (Figure 2).

Six years after planting, when the forest vegetation structure was evaluated, in the Siberian elm forest shelterbelt in Gradiștea, the number of trees per hectare decreased by 26% compared to that at planting, a lower percentage than in the Perișoru forest shelterbelt (Mihăilă et al., 2024). This may have several explanations: i) at Grădiștea, the forest shelterbelt was installed only with Siberian elm, therefore, there was no competition between species as in the Perișoru forest shelterbelt; ii) the seedlings adapted better to the seasonal conditions; and iii) the seedlings benefited from the groundwater supply during times of active growth, the Grădiștea shelterbelt being located near the Danube, at approximately 500-700 m. Because there were portions in the shelterbelt with a large number of trees, most of them with 2-5 shoots, maintenance work was necessary to direct the competition process between specimens and create space for the remaining specimens to grow.

Regarding the biometric characteristics 6 years after planting, the average diameter in the sample areas varies from 5.2 to 6.5 cm, and the average height from 4.8 to 6.4 m. Since the trees were very well developed, with the dominant height being over 8.5 m, the silvicultural work required in this shelterbelt can be considered the first thinning, even if no other care work was carried out up to this age. The purpose of this work is to create an optimal development space for the remaining trees and accelerate their height growth.



a.



b.

Figure 2. Forest shelterbelt with Siberian elm and honey locust in which no maintenance work was done (a) and after clearing work (b)

The oak and flowering ash forest shelterbelt from Grădiștea consists of five rows of trees arranged according to the scheme: STB • MJ STB • MJ (PĂ) • STB (STB - greyish oak, MJ - flowering ash, PĂ - wild pear). Unlike the other areas analysed, in the greyish oak and flowering ash shelterbelt, many specimens were missing in the rows with the flowering ash, in some cases, these species were completely missing, the cause being very likely the poor quality of the seedlings used for planting. The distance between rows of 2 m and between seedlings in a row of 1 m led to the development of greyish oak, which did not

have to endure the competition of flowering ash. At the time of the inventory, 6 years after planting, the total number of trees per hectare decreased by 45% compared to the planting date, the greyish oak by 30%, and the flowering ash by 37% (Mihăilă et al., 2024). The cleaning works carried out practically reduced the density in the greyish oak and flowering ash rows by extracting shoots and trees with negative characteristics and, thus, creating a larger space for the remaining trees to develop (Figure 3).



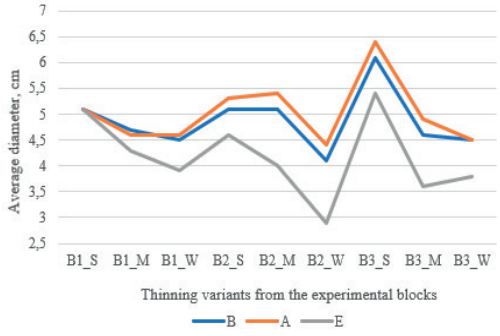
a.



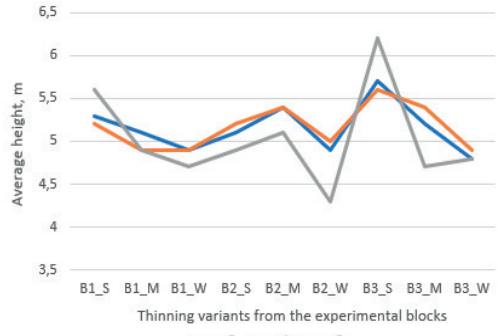
b.

Figure 3. Forest shelterbelt with greyish oak and flowering ash from Grădiștea, without tending operations (a) and after cleaning (b)

In all three forest shelterbelts, multiple shoots from a specimen were first extracted, with smaller dimensions than the remaining tree, small trees (both diameters and heights) that do not have the chance to develop very much, with wounds from maintenance work, injured by game species with sinuous trunks, bent, generically speaking, trees of inappropriate size and quality, which hamper the harmonious development of specimens with positive characteristics (straight, with symmetrical, developed crown) (Figure 4).



a.



b.

Figure 4. Variation of average diameter (a.) and average height (b.) in experimental blocks (B1, B2 and B3) before (B) and after cleaning works (A) and for extracted trees (E) (thinning variants are: S - strong, M - moderate, S - weak) in experimental setup installed in the Siberian elm and honey locust forest shelterbelt Perișoru

It was taken into account that the extractions carried out did not reduce the consistency below 0.8-0.7, which means not to strip the soil too much.

Regarding the intensity of tending operation after the basal area (i_{BA}), this naturally differed from one area to another, depending on the density, the number of specimens and the condition of the specimens in the analysed sample area. Starting from the scale for assessing the intensity of tending operation (MMAP, 2022), a similar scale for assessing the intensity of present interventions is proposed, but with different ranges: one for the shelterbelt composed of Siberian elm and honey locust and the other for the shelterbelt with greyish oak and flowering ash (Table 1).

In the first case, the extraction of trees from the sample areas determined the division of the cleaning and thinning into weak (i_{BA} < 10%), moderate (i_{BA} = 10-18%) and strong (i_{BA} > 18%). In the second case, the cleanings were of weak intensity, with i_{BA} < 5%, moderate, with i_{BA} = 5-8% and strong, with i_{BA} > 8%. It is mentioned that the establishment of these degrees is based only on the experiments in the three forest shelterbelts analysed and is not definitive.

Table 1. Intensity of tending operations and the ranges of variation of the three degrees of intensity (weak, moderate, strong)

Forest shelterbelt	Intensity of tending operations		
	Week	Moderate	Strong
	i_{Ba} < 10 %	**i_{Ba} = 10-18 %**	**i_{Ba} > 18 %**
Perișoru (Siberian elm, honey locust)	4.4; 8.7	11.8; 13.3; 13.6; 16.9	18.3; 18.5; 23,0.
Grădiștea (Siberian elm, honey locust)	5.0; 8.5; 9.5	12.1; 15.4; 18.1	18.8; 22.0; 26.6
	i_{Ba} < 5 %	**i_{Ba} = 5-8 %**	**i_{Ba} > 8 %**
Grădiștea (greyish oak, flowering ash)	3.0; 4.1	5.3; 5.6; 5.6; 7.3	8.5; 14.3

The maintenance works carried out, including cleaning and thinning, were performed during the growing season after the period of maximum accumulation specific to the beginning of the trees' vegetative phase, using lightweight mechanical saws and/or axes, by cutting the trees at ground level, which were then removed to the edge of the shelterbelt for utilization.

Regarding the effect of the maintenance works on the forest vegetation, it will be analysed after at least two - three growing seasons, the only year since the interventions did not show a significant response in terms of increases in height and diameter at 1.30 m.

For example, the analysis of relative height growth in the control and the three thinning variants at the Perișoru forest shelterbelt shows that the cuttings did not influence height growth. In the control variant, the height growth of the Siberian elm is higher, even significantly, compared to the moderate and strong thinning variants (Figure 5). In honey locust, the greatest height growth is recorded in the strong variant, although there are no significant differences between the four variants. This can be explained by the fact that height growth is greater under competition conditions between individuals.

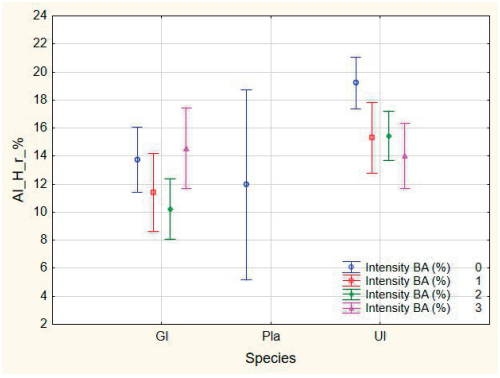


Figure 5. Relative height (H) growth of species in the Perisoru forest shelterbelt in the control variant (0) and the three thinning variants (1 - weak, 2 - moderate, 3 - strong) (Ul - elm, Gl - honey locust)

There are significant differences in diameter growth between the control variant and the three thinning variants, but only for the elm (Figure 6). This can be explained by the growth characteristics of the elm and the reduction in stand density, which led to increased growth primarily in diameter.

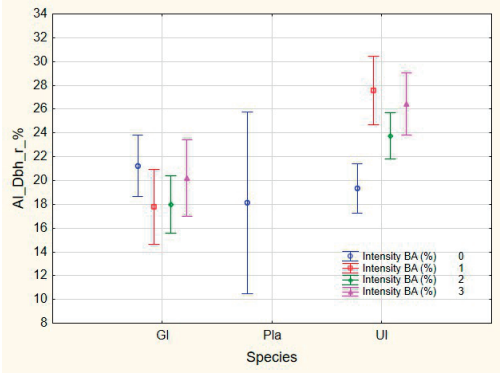


Figure 6. Relative diameter (Dbh) growth of species in the Perisoru forest shelterbelt in the control variant (0) and the three thinning variants (1 - weak, 2 - moderate, 3 - strong) (Ul - elm, Gl - honey locust)

At the Grădiștea forest shelterbelt, there are no significant differences between the control variant and the three thinning variants, neither for greyish oak nor for flowering ash (Figure 7).

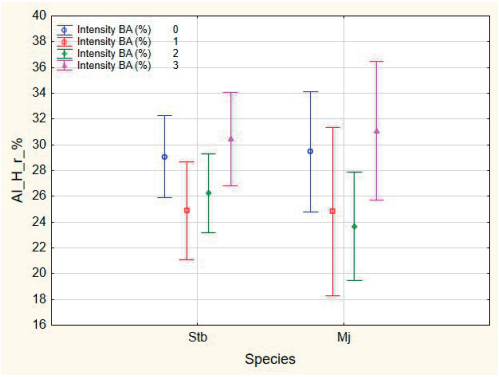


Figure 7. Relative height (H) growth of species in the Gradiștea forest shelterbelt in the control variant (0) and the three thinning variants (1 - weak, 2 - moderate, 3 - strong) (Stb - greyish oak, Mj - flowering ash)

Relative growth in diameter and height was analysed because, unlike absolute growth, it can better reflect differences in growth between species, considering that the shelterbelts consist of two to three different species. Additionally, growth differences also arise from the fact that after planting, supplementary plantings were carried out with seedlings that were generally smaller than those initially planted, leading to differences in tree sizes. Under these conditions, relative growth provides a clearer perspective on growth efficiency concerning the initial size of the trees.

CONCLUSIONS

Since, in the forest shelterbelts for crop protection, tending operations are not systematically performed, cleaning and thinning works were carried out, with different degrees of tree extractions, in three experimental setups installed in forest shelterbelts of different ages and compositions. In each experimental setup, three experimental blocks were placed, each block comprising four variants, one control and three variants with tree cutting/extractions of different intensities: strong, moderate and weak. The intensities of cleaning and thinning varied according to the condition of the forest shelterbelts (density and health of the trees).

The tending operations were carried out to create an optimal space for the development of the trees so that they develop their crown and grow in height, to protect the agricultural land to the greatest extent possible. The interventions had different intensities precisely to observe the best reaction of the trees to the tending operations while maintaining an adequate degree of semi-penetrability (40-60%) of the forest shelterbelts for field protection.

ACKNOWLEDGEMENTS

This research work was carried out with the support of Ministry of Education and Research (former Ministry of Research, Innovation and Digitalisation) through the national project PN23090203: New scientific contributions for sustainable management of watersheds, degraded lands, forest shelterbelts and other agroforestry systems in the context of climate change.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

METHODOLOGY AND EVALUATION CRITERIA OF THE CONSERVATION STATUS OF GRASSLAND HABITATS OF COMMUNITY INTEREST IN ROMANIA

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Abstract

For Romanian natural or semi-natural grassland habitat of Community interest assessed in all five terrestrial bioregions, the conservation status assessment activities were carried out based on the objectives of the EU Biodiversity Strategy for 2030, which is part of the European Green Deal. In our study we developed methods and used multicriteria analysis for all 2114 plots installed in 15 types of grassland habitats from the Natura 2000 sites of Community interests from Romania. In the studies conducted in the last five years, we evaluated the specific intensity of the pressures, followed by the estimation of the intensity of threats, affecting both the target habitats and their characteristic species. In order to compare the general trend of the conservation status in a biogeographic region, we established the trends within the Natura 2000 network. We realized the assessment matrix that uses information about the magnitude of short-term trends to assess the conservation status. Only stable or increasing trends can lead to a general conclusion regarding the favourable conservation status of the analysed habitat. Romania needs to effectively implement conservation measures for grasslands habitats.

Key words*: methodology, conservations status, grasslands habitats, community interest, Romania.*

INTRODUCTION

The *EU Biodiversity Strategy for 2030* (E.C., 2020) is part of the *European Green Deal* (E.C., 2019a; 2019b).

This strategy has an important point related with protecting and restoring nature in the European Union (EU) and should be done by improving and widening specified key commitments by 2030, define by the European Commission:

1. Legally protect a minimum of 30% of the EU's land area and 30% of the EU's Sea area and integrate ecological corridors, as part of a true Trans-European Nature Network.
2. Strictly protect at least a third of the EU's protected areas, including all remaining EU primary and old-growth forests.
3. Effectively manage all protected areas, defining clear conservation objectives and measures, and monitoring them appropriately.

Thus, three main commitments are foreseen for the network of protected areas by 2030, one of which also refers to natural or semi-natural grassland habitats.

For the *EU Biodiversity Strategy for 2030* to be effective, implementation of measures has to be significantly increased compared with the EU Biodiversity Strategy to 2020. At both national and international level, Habitats Directive (92/43/EEC) is the foundation of nature conservation in Europe and the development of EU Biodiversity Strategy for 2030 (EC, 2020).

The targets address the main drivers of biodiversity loss and aim to reduce key pressures on nature and ecosystem services in the EU. The strategy offers a great opportunity to halt or reverse biodiversity decline. The data obtained from assessment/ monitoring of the biodiversity in the years to come will be the foundation for achieving the goals of the 2030 Global Biodiversity Framework (EEA, 2020).

The *European Green Deal* sets out how to make from Europe the first climate-neutral continent by 2050, boosting the economy, improving people's health and quality of life, caring for nature, and leaving no one behind. At the same time, the European Green Deal priorities include protecting our biodiversity and ecosystems (EC, 2019b).

The EU implements policies and legislative frameworks (EU, 2025) for nature protection through the *Habitats Directive* (Directive 92/43/EEC) and the *Birds Directives* (Directive 79/409/EEC) adopted in 1979 and amended in 2009 (Directive 2009/147/EC).

Natura 2000 is the largest coordinated network of protected areas in the world. Established in 1992 to safeguard Europe's most valuable and threatened species and habitats, its area has steadily increased. In 2022, there were 27,193 Natura 2000 sites, covering 18.6% of the EU terrestrial and 9% of its marine territories. Effective management of these sites is the key for achieving Natura 2000's conservation aims. Member States still need to make significant efforts to establish and effectively implement conservation measures and management plans (Bendali & Nellas, 2016; EEA, 2024).

The total area of Natura 2000 sites network shows progress after protecting sites under the EU Habitats and Birds Directives in the EU, since 1993 to 2022 (Figure 1).

Data gathered for the period 1993-2019 shows the total protected area for EU-27 plus the United Kingdom. After this period, data collected from 2020 onward shows only the area for EU-27. The withdrawal of the United Kingdom from the EU led to a decrease in the Natura 2000 total area (EEA, 2024).

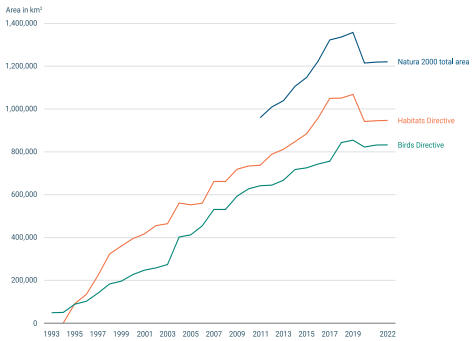


Figure 1. The total area of Natura 2000 sites` network in the European Union (EEA, 2024)

In the *Habitats Directive*, monitoring of Annex 1 habitats is required in Article 11 and into the Article 17 requires reporting every six years, for each Member State of the EU. All Member States reported in the mentioned periods and the national reports with the assessments at biogeographical level done by the ETC/BD (2014) and the EEA at the Article 17 web tool (Art17, 2025).

The EU habitats evaluation was based on the guidelines which have been compiled by the European Environment Agency (EEA) and its European Topic Centre on Biological Diversity (ETC/BD) and subsequent European Topic Centre on Biodiversity and Ecosystems (ETC BE). They have been developed through a collaborative work of the Expert Group on Reporting under the Nature Directives, its ad-hoc groups, the Expert Group on the Birds and the Habitats Directives (NADEG) and the Habitats Committee (DG Environment, 2017; 2023).

Description of the European habitats available for the reporting period 2007-2012 is included in scientific reference document *Manual of European Union Habitats* (EUR27, 2007; Evans & Arvela, 2011), and was updated for the period 2013-2018 (EUR28, 2013; C.E., 2024).

In the framework of European habitat types formal definitions, it had been developed a tool for assigning vegetation-plot records to the habitats from EUNIS (the European Nature Information System) (EEA, 2019). This system is used to classify a European vegetation-plot database, and compile statistically-derived characteristic species combinations and distribution maps for these habitats (Chytrý et al., 2020).

Bonari et al. (2023) stipulated that, with the knowledge gained after so many years working on habitats, Annex I of the Habitats Directive should be updated for solving ambiguities in the definition of Annex 1 habitat types.

The analysis of the Member States national reports revealed very different approaches and progress among Member States in developing and implementing monitoring programmes adapted to the reporting obligations laid down in Article 17 of the Habitats Directive. Following the 2013 report, the methods used for monitoring were analysed. It was found that the sample-based method is widely used, but the data collection, sample sizes and level of statistical certainty differ considerably.

To improve the comparability of results obtained by all Member States in the future from assessments of a habitat type, the

rapporteurs should follow simple minimum requirements regarding sample size and assessment methods for biogeographical regions (Ellwanger et al., 2018).

In biogeographic context for Romania, following Annex I of Habitats Directive, habitats and sites are areas whose conservation requires designation of special areas of conservation (SAC, SCIs). Also, Romanian habitat types were integrated into the Natura 2000 network (Doniţă et al., 2005; 2006; Gafta & Mountford, 2008; Strat et al., 2018; Ursu, 2020).

In the last 12 years, all Member States, including Romania, has reported two times to the Commission: in 2013 (for the evaluation period 2007-2012) and 2019 (for the evaluation period 2013-2018), based on every 6 years of the assessed conservation status for habitats of community interest (S.O.P., 2019; L.I.O.P., 2023).

At the European level there are big differences between the reporting of different countries. For example, it is difficult to compare Ireland with Romania. Even though there are recent publications on The Status of EU Protected Habitats and Species in Ireland (Lynn & O'Neill, 2019a; 2019b; 2019c). The results from reporting under the nature directives 2007-2019 were included in two reports of the European Environment Agency (EEA, 2015; EEA, 2020) very useful for comparing data from two reporting periods. Both reports included in particular information concerning the conservation measures referred to in Article 6(1) as well as evaluation of the impact of those measures on the conservation status of the natural habitat types from Annex I and the main results of the surveillance referred to in Article 11 (H.D., 1992).

MATERIALS AND METHODS

The assessment activities for establishing the conservation status were carried out taking into account the objectives of the European Union (EU) legal frameworks, strategies and action plans to protect nature and restore habitats and species, in the framework of SOP (2019) and LIOP (2023) projects financed by the Ministry of Investments and European Projects, through the Ministry of Environment, Water and Forests and implemented by the academic institutions in response of Romania to requests at the European Union obligations.

The analyses are related to the evaluation of the conservation status assessment was carried out for each type of grassland habitat present in Romania with reporting being carried out at national level, for all five biogeographical regions: ALP = Alpine, BLS = Black Sea (formerly 'Pontic'), CON = Continental, PAN = Pannonian, and STE = Steppe (Table 1).

Table 1. The grassland habitats distribution by regions in Romania

Habitat Natura 2000 code	Description	Region				
		ALP	BLS	CON	PAN	STE
6110	Rupicolous calcareous or basophilic grasslands of the *Alysso-Sedion albi*	+		+		
6120*	Xeric sand calcareous grasslands		+	+		+
6150	Siliceous alpine and boreal grasslands	+				
6170	Alpine and subalpine calcareous grasslands	+				
6190	Rupicolous pannonic grasslands (*Stipo-Festucetalia pallentis*)	+		+		
6210*	Semi-natural dry grasslands and scrubland facies on calcareous substrates (*Festuco-Brometalia*) (* important orchid sites)	+		+		
6230	Species-rich *Nardus* grasslands, on siliceous substrates in mountain areas (and submountain areas in Continental Europe)	+				
6240*	Sub-Pannonic steppic grasslands			+		
6260*	Pannonic sand steppes					+
62C0*	Ponto-Sarmatic steppes			+		+
6410	*Molinia* meadows on calcareous, peaty or clayey-silt-laden soils (*Molinion caeruleae*)	+	+	+	+	+
6420	Mediterranean tall humid grasslands of the *Molinio-Holoschoenion*		+			
6430	Hydrophilous tall herb fringe communities of plains and of the montane to alpine levels	+	+	+	+	+
6440	Alluvial meadows of river valleys of the *Cnidion dubii*	+	+	+	+	+
6510	Lowland hay meadows (*Alopecurus pratensis, Sanguisorba officinalis*)	+	+	+	+	+
6520	Mountain hay meadows	+				

Indicated by an asterisk (*) represent priority natural habitat types. In the sense of the Habitats Directive means natural habitat types in danger of disappearance, which are present on the territory referred to in Article 2 and for the conservation of which the Community has

particular responsibility in view of the proportion of their natural range which falls within the territory referred to in Article 2 (Habitats Directive, 1992).

The reporting format under Article 17 of the Habitats Directive for the period 2019-2024 has been modified. It contains technical documents related to reporting datasets, reporting tools and data specifications. The *Range tool* has been tested for the changes that have occurred and Member States have been invited to test it (last update 25.06.2024). The distribution of habitats in Romania according to their presence in the biogeographical regions has been established in accordance with the *Checklists for habitat types in Annex I* (EIONET, 2025).

In order to compare the general trend of the conservation status in a biogeographic region, we established the trends within the Natura 2000 network.

The data and observations have been collected from the field in 2114 plots distributed in all biogeographic regions. In our study, we used multi-criteria analysis for 15 grassland habitat types present in Natura 2000 sites of Community interest from Romania.

Before monitoring the habitats in the field, we establish an important aspect related to the surface of each plot/habitat type (Table 2).

The habitat inventory was carried out following a methodology based on the European Union Manual for the Interpretation of Natural Habitats (EUR 28, 2013) and other relevant publications for vegetation classification starting with the principles developed by Braun-Blanquet (1964) and up to the applied science of vegetation, conservation planning and land management developed in the hierarchical system of floristic classification in Europe (Mucina & al., 2016). This methodology was correlated with the description of Romanian habitats (Doniță & al., 2005, 2006; Gafta & al., 2008), as well as with the Romanian habitat monitoring guide (E.W.F.M.O, 2023), and applied in "itinerary" and "stationary" investigations, in two stages: the analytical stage and the synthetic stage. The evaluation method was established for the monitoring plot.

The distribution and area maps of each habitat type will be produced in the standard 10x10 km ETRS format, ETRS 5210 projection. According to EC instructions, the use of attribute data to indicate the presence of a habitat in a grid cell is not allowed. The distribution maps will be completed with the necessary metadata, in accordance with EC requirements, and in accordance with the Infrastructure for Spatial Information in Europe (INSPIRE, 2007) specifications, when these will be available for these types of data. The metadata will contain, among other things, the period in which were collected distribution data, projection system, date, etc.

By identifying the key species (characteristics) of the habitats of community interest, determinations were made using variables related to ecosystem functions, such as: optimal monitoring period, qualitative and quantitative structure of the identified habitat and identified flora species. The data collected from the field will be entered in the monitoring sheets.

The data aggregation method is done by: (1) spatial aggregation, respectively generating maps in accordance with the reporting format starting from the evaluated (and estimated) plots, synthesizing at the bioregion level and respectively at the national level; (2) non-spatial aggregation which involves generating specific files, in accordance with the reporting format, starting from the primary aggregated data; (3) national aggregation level of attributes at the national level resulting from the aggregation of data at the bioregion level.

The second level of aggregation is for the attributes specific to the target habitats at plot level which are then correlated at bioregion level for reporting.

The information from the 10 x 10 km grids (plots) at bioregion level is averaged for each category of status parameters using a weighted average to make the final decision. For example, the specific composition which is one of the essential parameters in assessing habitat structure will have different values from one plot to another, which requires that from a systematic point of view they be grouped into different species richness classes (high, medium, low); the weight of each class being the one that will lead to the decision specific to each bioregion. Thus, for example, if in habitat 6110 we obtain 23 plots that fall into the high specific composition class (12 characteristic species), and in the medium composition class

(7 characteristic species) we will have 7 plots that fall into this, then into the low composition class (3 characteristic species), we can deduce that the report on the target bioregion is considered in a favourable state in terms of structure assessment.

Direct observations assessed by biodiversity experts who assessed the target habitats used state-of-the-art technologies in terms of both GPS (Global Positioning System) units and mobile applications for field data collection. The completed field sheets described structural and functional characteristics of the habitats, as well as identified pressures. The information thus collected entered a first aggregation process (first level) in which each sheet has an associated stationary location with metric precision (1-3 m) and is aggregated into a 10 x 10 km plot. This aggregation allows the characterization of the internal variability of the target habitat at the level of a plot. The plot represents the basic unit for assessment. The assessment methods are carried out either by a complete assessment of the target habitat (3 = Complete survey or a statistically robust estimate); when a number of plots smaller than the total number in which we have the confirmed habitat is assessed (2 = Estimate based on partial data with some extrapolation and/or modelling); when a very small number of plots are evaluated and expert opinion is used (1 = Estimate based on expert opinion with no or minimal sampling); when no plots are evaluated in the report, "data not available" will be mentioned (0 = Absent data).

For the upcoming reporting in July 2025, we also used the European framework (DG Environment, 2023; C.E., 2024).

To explain the assessment of habitats of community importance, we used the following attributes as defined in the Explanatory Notes and Guidelines of the Habitats Directive (DG Environment, 2023): *Range (and Surface Area), Habitat structure, Pressures (and threats and conservation measures) and Future Prospects,* all assessed separately for each biogeographical region (Mihăilescu et al., 2024).

The assessment matrix had been realized using information about the magnitude of short-term trends to assess the conservation status. Only stable or increasing trends can lead to a general conclusion regarding the favourable conservation status of the habitat analysed. Romania needs to effectively implement conservation measures for grasslands habitats.

The framework for assessing the sustainability of natural resource management systems is organized on the attributes of productivity, stability, reliability, resilience, adaptability, equity, and self-reliance (Barron et al., 2021).

Table 2. The grasslands habitat types and the size of their sample areas used in grassland habitats monitoring

Habitat Natura 2000 code	Habitat area	
	<1 ha	>1 ha
	Plot surfaces	
6120* Xeric sand calcareous grasslands	1x1 m; 5x5 m; 10x10 m	5x5 m; 10x10 m
6150 Siliceous alpine and boreal grasslands	1x1 m; 5x5 m; 10x10 m	5x5 m; 10x10 m
6170 Alpine and subalpine calcareous grasslands	1x1 m; 1x10 m; 5x5 m; 10x10 m	5x5 m; 10x10 m
6190 Rupicolous Pannonic grasslands (*Stipo-Festucetalia pallentis*)	1x1 m; 1x10 m; 5x5 m; 10x10 m	5x5 m; 10x10 m
6210* Semi-natural dry grasslands and scrubland facies on calcareous substrates (*Festuco-Brometea*) (* important orchid sites)	1x1 m; 1x10 m; 5x5 m; 10x10 m	2x10 m 5x5 m; 10x10 m
6230* Species-rich *Nardus* grasslands, on siliceous substrates in mountain areas (and sub-mountain areas, in Continental Europe)	1x1 m; 1x10 m; 5x5 m; 10x10 m	5x5 m; 10x10 m
62C0* Ponto-Sarmatic steppes	5x5 m; 10x10 m	10x10 m
6240* Sub-pannonic steppic grasslands	5x5 m; 10x10 m	10x10 m
6260* Pannonic sand steppes	1x1 m; 1x10 m; 5x5 m; 10x10 m	5x5 m; 10x10 m
6410 *Molinia* meadows on calcareous, peaty or clayey-silt-laden soils (*Molinion caeruleae*)	1x1 m; 1x10 m; 5x5 m; 10x10 m	5x5 m; 10x10 m
6420 Mediterranean tall humid herb grasslands of the *Molinio-Holoschoenion*	5x5 m; 10x10 m	5x5 m; 10x10 m
6430 Hydrophilous tall-herb fringe communities of plains and of the montane to alpine levels	5x5 m; 10x10 m	5x5 m; 10x10 m
6440 Alluvial meadows of river valleys of the *Cnidion dubii*	1x1 m; 1x10 m; 5x5 m; 10x10 m	5x5 m; 10x10 m
6510 Lowland hay meadows (*Alopecurus pratensis, Sanguisorba officinalis*)	1x1 m; 1x10 m; 5x5 m; 10x10 m	5x5 m; 10x10 m
6520 Mountain hay meadows	5x5 m 10x10 m	10x10 m

An important instrument was used and it is represented by the Article 17 web tool on biogeographical assessments of conservation

status of species and habitats under Article 17 of the Habitats Directive. This instrument gives access to assessments at Member State biogeographical level done by Member States and at EU biogeographical level done by the ETC/BD and the EEA (Art17, 2025) and it can be used by all Member States for comparing assessment of national and EU level.

The concept of favourable reference values is derived from definitions in the Directive, and relates to the "long term natural distribution, structure and functions as well as the long-term survival of its typical species" (Article 1(e)) in their natural range.

Overall assessment of conservation status uses four categories: "favourable" (FV), "unfavourable - inadequate" (U1), "unfavourable - bad" (U2) and "unknown" (XX), based on the evaluation matrix for assessing conservation status for a habitat (Table 3).

Table 3. Overall assessment of the conservation status (CS)

Status of parameters	All favourable or few favourable + one unknown	One or more inadequate, but no bad	One or more bad	Two or more unknown +favourable or all unknown
Overall assessment of CS	Favourable (FV)	unfavourable-inadequate (U1)	unfavourable-bad (U2)	Unknown (XX)

RESULTS AND DISCUSSIONS

For reporting to the EC, Romania followed the Reference portal for reporting under Article 17 of the Habitats Directive and created the guide regarding the protocols and unitary methodologies for monitoring the conservation status of community interest habitats.

For the reporting due in July 2025, an updated guideline has recently been introduced into Romanian legislation (E.W.F.M.O, 2023) and must be followed by all scientists reporting to the European Union on the conservation status of habitats.

Threats and pressures for all habitats can be selected from the reference portal for reporting under Article 17 of the Habitats Directive, usually the most relevant and important ≤10.

Romania is currently in its third report under Article 17 of the Habitats Directive.

Data resulting from the monitoring should capture where the main objective of the conservation action need to take place.

The identified EU interest habitats on the Romanian territory showed that they might be distributed in more than one biogeographic region (Table 4). Even the conservation status of some was unknown during the first reporting period (6120* for the BLS and STE regions), new assessments realized during the other two reporting periods revealed their conservation status. For instance: the habitat 6120* Xeric sand calcareous grasslands from Steppe biogeographical region (STE) had "unknown" status during first reporting period. Also, some of the habitats (6430, 6440, 6520) present different conservation status in time in the same biogeographic region.

Table 4. Romanian national report under Art. 17 of the Habitats Directive

Habitat Natura 2000 code	RO national report		
	2013 (period 2007-2012)	2019 (period 2013-2018)	2025 (period 2019-2024)
6110	ALP	ALP	ALP
6110	CON	CON	CON
6120*	N/A	BLS	BLS
6120*	CON	CON	CON
6120*	N/A	STE	STE
6150	ALP	ALP	ALP
6170	ALP	ALP	ALP
6190	ALP	ALP	ALP
6190	CON	CON	CON
6210*	ALP	ALP	ALP
6210*	CON	CON	CON
6230	ALP	ALP	ALP
6240*	CON	CON	CON
6410	ALP	ALP	ALP
6410	BLS	BLS	BLS
6410	CON	CON	CON
6410	PAN	PAN	PAN
62C0*	CON	CON	CON
62C0*	STE	STE	STE
6420	BLS	BLS	BLS
6430	ALP	ALP	ALP
6430	BLS	BLS	BLS
6430	CON	CON	CON
6430	PAN	PAN	PAN
6430	STE	STE	STE
6440	ALP	ALP	ALP
6440	BLS	BLS	BLS
6440	CON	CON	CON
6440	PAN	PAN	PAN
6440	STE	STE	STE
6510	ALP	ALP	ALP
6510	BLS	BLS	BLS
6510	CON	CON	CON
6510	PAN	PAN	PAN
6510	STE	STE	STE
6520	ALP	ALP	ALP

For the reporting period 2007-2012, for 15 assessed grasslands habitats indicated that their conservation status related to the 34 evaluations on biogeographic distribution were: unfavourable-bad (1 habitat), unfavourable-inadequate (4 habitats), favourable (29 habitats), and unknown (0 habitats) (Mihăilescu et al., 2015).

For the reporting period 2013-2018, the number of evaluations increased at 37, thus conservation status has been: unfavourable-bad (1 habitat), unfavourable-inadequate (4 habitats), favourable (33 habitats), and unknown (0 habitats).

For the upcoming reporting in July 2025 (reporting period 2019-2024), we have prepared the assessment of the conservation status of habitats of community interest for grassland. Thus, we propose from 15 assessed habitats, the conservation status related to the 37 evaluations on biogeographic distribution as it follows: unfavourable-bad (1 habitat), unfavourable-inadequate (7 habitats), favourable (29 habitats), and unknown (0 habitats).

These assessments shown that for some habitats (6430, 6440, 6520), in certain biogeographic regions, a large decrease in area has occurred: equivalent to a loss of more than 1% per year. In these unfavourable-bad or unfavourable-inadequate assessed cases, the indicative value of the country may deviate from the favourable status in the period 2019-2024.

The conservation status of most of the grasslands' habitat is stable during three reporting periods (2013, 2019, 2025).

Decreasing trends in the conservation status from favourable (FV) to unfavourable-inadequate (U1), due to increasing trends in the intensity of different impacts upon the following habitats: 6430 (Hydrophilous tall-herb fringe communities of plains and of the montane to alpine levels), 6440 (Alluvial meadows of river valleys of the *Cnidion dubii)* and 6520 (Mountain hay meadows).

For habitat 6240 in the CON region, the decrease in the range surface/area of distribution radius was estimated at 8400 ha, and for habitat 6420 in the BLS region, the decrease in the range surface/area of distribution radius was estimated at 800 ha, in conclusion, the assessment was established at U1.

The assessment of grassland habitats was carried out using a model-based and reference-based approach (DG Environment, 2023). The results were interpreted on three generic levels of data availability and knowledge and are suggested to be interpreted in three ways:

- *High*: good data on actual distribution and ecological requirements/features; good historical data and trend information;
- *Moderate*: good data on actual distribution and ecological requirements/features; limited historical distribution data (only trend data available);
- *Low*: data on actual distribution and ecological requirements/features are sparse and/or unreliable; hardly any historical data available and no trend information.

Conservation measures are taken inside or outside Natura 2000 sites for each habitat type: a) maintaining its current range, surface area or structure and functions; b) expanding its current range; c) increase its surface area; and d) restore the structure and functions, including the status of typical species.

Recently regarding the multidisciplinary and multiscale approach sustainability assessment of pasture, two holistic indicator-based frameworks for livestock sustainability assessment include this multidimensionality and multifunctionality aspects. The sustainability assessment of farming and the environment framework includes the three classical pillars of sustainability and is structured on content-based principles, criteria, and indicators (Barron, et al., 2021).

Future Prospects indicate the expected direction of change in conservation status in the near future based on a consideration of current status.

The next step was to evaluate the future prospects of each parameter, one after the other, correlated in the matrix. (Table 5).

Table 5. Assessing the impact of reported threats using scope and influence (After: DG Environment, 2023)

Scope	Influence		
	High	Medium	Low
Whole (>90%)			
Majority (50-90%)			
Minority (<50%)			

Legend: High impact | Medium impact | Low impact

Trends are an essential part of assessing all conservation status parameters except Future prospects. A comparison between the overall trend of habitat area and the area in good condition in the biogeographical or marine region and trends within Natura 2000 is important in assessing the impact of the Natura 2000 network on conservation status.

The trend is described using qualitative indicators such as: stable, recovering, declining and unknown. Evaluation of short-term trends is also the key control for the quality analysis of successive reporting results. In order to establish pressures and threats, we used the nomenclature standardly applied at the European level.

For some habitats (6240*, 62C0*, 6410, 6420, 6430, 6440, 6520), the main pressures are related to: (1) the land use changes that occurred during the reporting period but it refers to instances where continuing the agricultural use of originally non-agricultural habitat prevent the latter from being restored (e.g. in case of land use conflicts between agriculture and nature conservation), (2) intensive grazing or overgrazing by livestock and, (3) the development renewable energy infrastructures, as follow:

PA01 - Conversion of natural and semi-natural non-agricultural habitats (e.g. semi-natural forests) and non-agricultural habitats of species targeted by the nature directives into agricultural land (e.g. pastures, meadows, arable fields).

PA04 - Use of plant protection chemicals in agriculture (e.g. pesticides, fungicides, etc.).

PA07 - Intensive grazing or overgrazing by livestock in agricultural and agroforestry habitats (e.g. pastures, meadows, pastured forests) where grazing causes damage to vegetation or soil (e.g. trampling, nitrogen input) or where livestock presents a disturbance or a competitor for species targeted by the nature directives. Includes intensive grazing in other habitats in agricultural landscapes (riparian areas, bogs, scrubs and forests) where grazing causes damage to vegetation or habitats. Also included are situations due to inadequate or lack of conservation management.

PA19 - Agricultural activities generating soil pollution

PD01 - Renewable energy (wind, wave and tidal power) generation including development and use of associated infrastructure (e.g. building wind turbines or tidal barrages).

PD03 - Renewable energy (solar power) generation including development and use of associated infrastructure (e.g. building solar farms).

Pressures observed in the field study shall be assessed by determining the specific intensity of each one exerted on the target habitat. The categories of intensity assessment are also qualitative i.e. low, moderate, high, unknown.

In the absence of dedicated monitoring schemes, trends are usually a result of expert opinion and in that case should be reported only as directions (increasing/decreasing/stable), without absolute values. Unknown trends should be reported as 'unknown'. If the available data are not sufficient to determine trend direction, this can be reported as 'uncertain'. It is therefore recommended to estimate short-term trend over two reporting cycles, i.e. 12 years (or a period as close to this as possible), as this should give a more reliable and comparable estimate of the trend (DG Environment, 2023).

For the third reporting, Romania must consider as the period for assessing trends the short-term one, considered after two reporting cycles (12 years; or a period as close as possible).

CONCLUSIONS

Assessment of trends and conservation status of grasslands habitats of community interest from Romania, has been developed using standard methodology based on four parameters as defined in Article 1 of the Habitats Directive. For reporting to the EC, Romania used methodology in accordance with Reference portal for reporting under Article 17 of the Habitats Directive and created the Guide regarding the protocols and unitary methodologies for monitoring the conservation status of community interest habitats.

The results obtained from data and observations collected from the field have a high scientific value and underpin all analyses and interpretations.

The conservation status of most of the grasslands habitat is stable during three

reporting periods (2013, 2019, 2025); only a few habitats (6430, 6440 and 6520) showed decreasing trends in the conservation status from favourable (FV) to unfavourable-inadequate (U1), due to increasing trends in the intensity of different impacts upon the habitats.
The first report provided the baseline for subsequent reports, including the assessment of short-term conservation trends over a single reporting cycle. The short-term nature of the data available so far means that the full assessment recommended by the standard EU methodology cannot yet be carried out.
Only stable or increasing trends can lead to an overall conclusion on the favourable conservation status of the habitat under consideration. Romania needs both to establish and effectively implement conservation measures for grassland habitats, and develop updated management plans for protected areas.
Romania is currently carrying out the third report under Article 17 of the Habitats Directive.
For the next report (deadline 31 July 2025), was approved in 2024 the reporting format on habitat types listed in Resolution No. 4 (1996) with a 1 to 1 relationship with habitats of Annex I of the EU Habitats Directive. In this way, it was possible to extent the format of the EU reporting under the Article 17 of the Habitats Directive and to benefit from the IT infrastructure developed by the European Environment Agency. Each of the new and amended existing fields of the Reporting Format 2019-2024 was presented with a short explanation and discussion on the adapted possibilities for non-EU Contracting Parties to motivate the exchange of views (C.E., 2024).

ACKNOWLEDGEMENTS

Data obtained in the framework of the Sectorial Operational Programme SOP-ENVIRONMENT 2007-2013 (SMIS-CSNR 17655) and the Large Infrastructure Operational Programme 2014-2020 (LIOP no. SMIS 2014+120009) projects financed by the Ministry of Investments and European Projects. The paper was written in the framework of projects RO1567-IBB01 and RO1567-IBB04 from Institute of Biology Bucharest of Romanian Academy financed by Romanian Academy.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

POTENTIAL HABITAT PREDICTION FOR THE CURRENT DISTRIBUTION OF *Tozzia alpina* subsp. *carpathica* IN THE ROMANIAN CARPATHIANS

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Abstract

Tozzia alpina subsp. carpathica is a rare semiparasitic plant that faces significant conservation concerns within its native range in the Carpathian Mountains of Romania. Currently classified as data deficient on the IUCN Red List, this species requires accurate habitat prediction to guide effective conservation strategies and deepen our understanding of the factors shaping its distribution. The conservation status assessment, conducted under Article 17 of the Habitats Directive, relies on presence points within 10x10 km squares. However, current estimates require a robust distribution model to assess habitat extent accurately. This plant is typically found in small patches within wet meadows and tall hydrophilous vegetation along springs and rivers in the alpine bioregion, making it vulnerable to land use and resource exploitation. Therefore, developing models to predict the habitat distribution is a priority. We employed the Maximum Entropy modeling approach to address this need and predict suitable habitats, utilizing species occurrence data in conjunction with environmental variables. This modeling method provides valuable insights for identifying areas to evaluate in the upcoming reporting period under the Habitats Directive, significantly enhancing conservation efforts.

Key words*: Carpathians, conservation, distribution, Maximum Entropy modelling, Tozzia alpina subsp. carpathica.*

INTRODUCTION

Species Distribution Models (SDMs) have become essential tools in ecology and conservation science, offering robust methods for estimating the potential geographic distribution of species based on known occurrences and associated environmental conditions (Austin, 2007; Elith & Leathwick, 2009; Guisan et al., 2017)

These models are particularly useful for rare, data-deficient, or ecologically specialized taxa, for which direct field observations are often spatially limited (Wang et al., 2015; Mousikos et al., 2021; Bao et al., 2022; Ramírez-Rodríguez et al., 2025). SDMs help identify priority areas for species monitoring, guide conservation action, and support ecological reporting under national and international frameworks. Their integration into biodiversity assessments has proven valuable for improving plant species conservation status evaluations, optimizing field survey design, and informing long-term habitat management strategies (Jansen et al., 2021). In the context of the Habitats Directive, SDMs are increasingly used to complement official range estimates and support reporting under Article 17 (Sousa-Silva et al., 2014).

Under Article 17, Member States assess the conservation status of species using presence data aggregated in 10 × 10 km UTM grid squares (M.O., 2023). However, this resolution and methodology, implemented through the Range tool provided by the European Commission, may significantly underestimate actual species distributions, particularly for plants with narrow ecological amplitudes or fragmented populations (Jansen et al., 2021). The coarse spatial scale and limited gap analysis functions reduce the ability to detect microhabitat-level patterns that are ecologically meaningful for habitat-restricted taxa. For species such as *Tozzia alpina* subsp. *carpathica* (Wołł.) Pawłł., which occurs in small, isolated populations confined to habitats like wet

subalpine meadows, hygrophilous tall-forb communities, or spring margins, this limitation can lead to underreporting of potential distribution and habitat suitability.

The genus *Tozzia* comprises a small group of hemiparasitic plants within the Orobanchaceae family, represented by two closely related taxa: *T. alpina* subsp. *alpina*, distributed from the Pyrenees to the western Balkans, and *T. alpina* subsp. *carpathica* is restricted to the Carpathians and the southern Balkan Mountains (Kliment, 1999; Sârbu et al., 2013; POWO, 2025). *T. carpathica* is a species of community interest (code 4116) listed under Annexes II and IV of the Habitats Directive (92/43/EEC) and under the Bern Convention (Resolution 6/1998). It is also classified as Data Deficient on the IUCN Red List (Bilz, 2011). However, it is considered nationally rare or threatened in countries such as Romania (Oltean et al., 1994), Poland (Wika et al., 2014; Kazmierczakowa et al., 2016), Slovakia (Mereďa & Hodalová, 2011), and Bulgaria (Petrova & Vladimirov, 2009; Vladimirov & Assyov, 2011).

In Romania, the species is included among the 90% of vulnerable Carpathian taxa covered by the national protected area network (Coldea et al., 2009). Out of the 45 Natura 2000 sites where the species has been documented across Europe, 23 are situated in Romania (EUNIS, 2025), primarily in montane and subalpine regions. Although considered well protected, the species' distribution remains fragmented and incompletely understood. Recent confirmations in protected areas such as ROSCI0225 Grădiștea Muncelului-Cioclovina (Anonymous, 2016) and ROSCI0122 Făgăraș (FCC, 2021) indicate that *T. alpina* subsp. *carpathica* may occur in additional, previously undocumented microhabitats, highlighting the need for targeted field surveys in suitable but underexplored areas.

This study hypothesises that the current distribution of *T. alpina* subsp. *carpathica* in Romania is underestimated due to the limitations of the standard reporting framework. To address this, we aim to develop a habitat suitability model using MaxEnt for the Romanian Carpathians. The objectives are: (1) to identify key environmental variables influencing habitat suitability; (2) to generate a predictive map of potentially suitable habitats; and (3) to provide spatial insights that can support future field validation, conservation planning, and reporting under the Habitats Directive.

MATERIALS AND METHODS

Study area

The study was carried out in Romania, a southeastern European country covering approximately 238,400 km² (EEA, 2020). Romania encompasses five biogeographical regions: Alpine, Continental, Pannonian, Steppic, and Black Sea, making it one of the most ecologically diverse member states of the European Union (Iojă et al., 2010; Evans, 2012). The Romanian Carpathians occupy about one-third of the national territory, being a hotspot of biodiversity and endemism, harbouring numerous relict and endemic species, particularly in alpine and subalpine habitats (Coldea et al., 2009; Biriş et al., 2011). The climate is temperate-continental, with four distinct seasons and significant regional variability, supporting a broad range of ecosystems from deciduous and coniferous forests to alpine grasslands and peatlands.

Biological characteristics

T. alpina subsp. *carpathica* is a perennial hemiparasitic plant with a distinct developmental cycle of two stages. In the initial phase, lasting up to 10 years, the plant persists underground as a rhizome with scale-like, non-photosynthetic leaves, relying entirely on its host for nutrients (McNeal et al., 2013). Only after this extended subterranean period does it emerge above ground, developing green leaves and transitioning into a hemiparasitic state, with all emerging shoots being fertile (Těšitel et al., 2010).

The plant is 10-50 cm tall, with a fragile, often branched stem that is four-angled and glabrous or sparsely hairy along the ridges; the leaves are opposite, sessile, ovate to broadly ovate, somewhat fleshy, glossy, and glabrous, with shallow coarse teeth (1-3) near the base; bracts are similar to the leaves but smaller; the inflorescence is a short, lax raceme borne at the ends of branches; the calyx is campanulate and weakly bilabiate; the corolla has five obtuse

lobes forming two barely distinct lips, and is small, yellow, and tubular (6-8 mm), with reddish speckles; the plant has four didynamous stamens with filaments fused to the corolla tube; the fruit is a globular, one-chambered capsule (2-2.5 mm) enclosed within the calyx, containing one or two seeds; flowering typically occurs from July to August, in the upper areas even until September (Paucă, 1960; Sârbu et al., 2013).



Figure 1. *Tozzia alpina* subsp. *carpathica* on a riverbank in an alluvial meadow, growing among *Petasites hybridus*, its host species (Photo Nicoară R., 25.05.2023)

The typical subspecies is most commonly associated with tall perennial herbaceous hosts from the genera *Cicerbita*, *Adenostyles*, and *Petasites* (Piękoś-Mirkowa, 2004). In Romania, the Carpathian subspecies has frequently been observed parasitizing species from the Petasites genus. This relationship plays a crucial role also in determining the species distribution. Since *Petasites* thrive in moist environments such as mountain valleys and near water sources, these habitats also become the primary locations where *T. alpina subsp. carpathica* can establish itself. The presence and abundance of the host plant directly influence the occurrence of *Tozzia*, highlighting the importance of suitable hydrophilous vegetation in its habitat selection.

Preferred habitat

In Romania, *T. alpina* subsp. *carpathica* is a mesohygrophyte and microthermophyte with a narrow ecological niche, occurring predominantly in humid montane and subalpine zones of the Carpathians (Sârbu et al., 2020). The species is highly shade-tolerant and hygrophilous, typically restricted to spring-fed habitats, streambanks, wet forest clearings, and tall-herb communities that maintain stable microclimatic conditions and elevated edaphic moisture (Figure 1).

It is most frequently associated with vegetation types belonging to the classes Betulo-Adenostyletea Br.-Bl. et R. Tüxen 1943, Molinio-Arrhenatheretea R. Tüxen 1937, and Montio-Cardaminetea Br.-Bl. et R. Tüxen 1943 (Sanda et al., 2008; Neblea & Dragomir, 2015). Within the Montio-Cardaminetea class, *T. alpina* subsp. *carpathica* occurs in spring-fed and streamside vegetation of the Montio-Cardaminetalia alliance, especially in communities such as *Cardamino–Chrysosplenietum alternifolii*, dominated by *Cardamine amara* and *Chrysosplenium alternifolium*. These vegetation types typically develop around mountain springs in beech (*Fagus sylvatica*) and spruce (*Picea abies*) forest belts, on both siliceous and calcareous substrates.

Within the Betulo-Adenostyletea class, the species is found in tall-herb communities occupying wet slopes, forest glades, and ravines between 900 and 1200 m altitude. These habitats thrive in conditions of high soil moisture and persistent atmospheric humidity. Here, *T. alpina* is considered characteristic of the Adenostyletalia alliariae order, particularly within the Adenostylion alliariae alliance - composed of hygrophilous tall-herb vegetation growing on nutrient-rich, moist colluvial soils. In these plant communities, it coexists with species such as *Adenostyles alliariae*, *Senecio nemorensis*, *Veratrum album*, *Rumex alpestris*, and *Salix hastata*.

Within the Molinio-Arrhenatheretea class, *T. alpina* subsp. *carpathica* is associated with the *Calthion palustris* alliance. It occurs in associations such as *Scirpetum sylvatici* Ralski

1931 emend. Schwich 1944 and *Chaerophyllo hirsute-Filipenduletum* Niemann et al. 1973, from the *Filipendulion* alliance (Lohmeyer in Oberdorfer et al., 1967), which develop on alluvial and pseudogley soils over siliceous rocks, typically in marshy habitats and along streams (Neblea & Dragomir, 2015).
Furthermore, the species has been recorded in several Natura 2000 habitat types characterized by consistently high soil moisture, including habitat types: 3220 Alpine rivers and the herbaceous vegetation along their banks, 4060 Alpine and Boreal heaths, 4070* Bushes with *Pinus mugo* and *Rhododendron hirsutum* (*Mugo-Rhododendretum hirsuti*), 6150 Siliceous alpine and boreal grasslands, 6170 Alpine and subalpine calcareous grasslands, and 6430 Hydrophilous tall-herb fringe communities of plains and of the montane to alpine levels (Doniţă et al., 2005; Gafta & Mountford, 2008; Drăgulescu, 2008; Brînzan, 2013; Neblea & Dragomir, 2015).

Reporting under Article 17

Monitoring species listed in Annex II and Annex IV of the Habitats Directive (92/43/EEC) is required under Article 11 and Article 17, preceding the reporting of species conservation status to the European Commission every six years. According to the Habitats Directive, measures must aim to maintain or restore species of wild flora of community interest to a favorable conservation status.
Over the past 12 years, all Member States, including Romania, have reported twice to the European Commission: first in 2013 (covering 2007-2012) and again in 2019 (covering 2013-2018). The next reporting deadline is 31 July 2025, with an updated reporting format for species types approved in 2023 (DG Environment, 2023).
The monitoring of the conservation status of plant species of community interest in Romania was structured around three key components. First, a review of all available data from scientific publications, herbarium records, and research or conservation projects were assessed in order to generate the initial inventories and distribution maps. Second, field data were collected to verify and add new data to existing records, supporting the development of comprehensive species inventories and GIS-based distribution maps. In the alpine bioregion, where *T. alpina* subsp. *carpathica* is distributed, monitoring was focused on assessing the species' range, population size and distribution, surface area of occurrence, trends in population dynamics, current conservation status, and major pressures and threats, both historical and ongoing. Finally, all data were analyzed and validated before being integrated into the national plant monitoring system.
Within these framework projects, carried out between 2011-2023, the *T. alpina* subsp. *carpathica* population was inventoried in Romania and the conservation status of this species was evaluated (Mihăilescu et al., 2015a). The species identification was carried out in the field, no specimens were collected.
The concept of favourable reference values is derived from definitions in the *Habitats Directive* (DG Environment, 2023).
Overall assessment of conservation status uses four categories: "favourable" (FV), "unfavourable - inadequate" (U1), "unfavourable - bad" (U2) and "unknown" (XX), based on the evaluation matrix for assessing conservation status for a species (Table 1).

Table 1. Overall assessment of the conservation status (CS)

Status of parameters	All favourable or few favourable + one unknown	One or more inadequate, but not bad	One or more bad	Two or more unknown + favourable or all unknown
Overall assessment of CS	Favourable (FV)	Unfavourable-inadequate (U1)	Unfavourable-Bad (U2)	Unknown (XX)

Table 2. Overall assessment of the conservation status on each reporting period

Species	2013	2019	2025
	ALP	ALP	ALP
4116 *Tozzia alpina* subsp. *carpathica* (Woll.) Pawł	FV	FV	FV

During 2007-2023, the conservation status was "favourable" (FV), due to increasing trends in the intensity of different impacts upon the species habitat (Table 2).
For the period 2013-2018 the population size measured in number of individuals was estimated between 10-400.
Under the Habitats Directive - Article 17 reporting process, these assessments have been

carried out in EU25 for the period 2001-2006, in EU 27 for the period 2007-2012 and in EU28 for the period 2013-2018 (EIONET, 2019). Romania has already reported in the last two periods. The next report is due on 31 July, 2025.

During 2007-2023, the conservation status was "favourable" (FV), due to increasing trends in the intensity of different impacts upon the species habitat (Table 2).

To generate the distribution maps, we used standard methods based on occurrence data reported under Article 17. Specifically, species occurrences were aggregated within standardized ETRS89 grids (10 x 10 km and 1 x 1 km resolution, projected in ETRS LAEA 5210 (M.O., 2023). Subsequently, the Art17 reporting tool was employed to derive the species' range distribution, using a gap analysis technique integrated into the reporting tool (Figure 2).

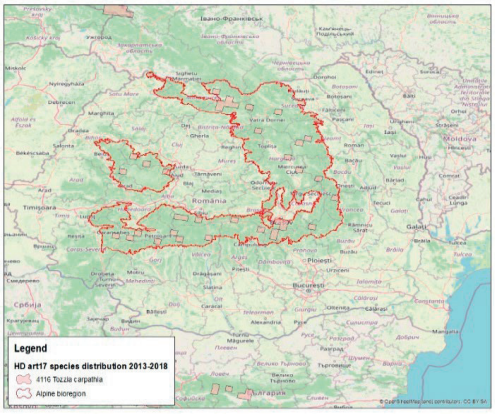


Figure 2. Habitat directive *Tozzia alpina* subsp. *carpathica* species distribution from 2013-2018 reporting period

Mapping and assessment of species state

The potential habitat distribution of *T. alpina* subsp. *carpathica* was modelled using the Maximum Entropy algorithm (MaxEnt), a species distribution modelling method especially effective for presence-only datasets and limited occurrence records. This machine-learning algorithm enabled predictions based on environmental variable similarities. Our aim was to assess whether the species distribution area previously estimated by the Article 17 reporting tool may have been underestimated.

A total of 37 validated presence plots were established by integrating distribution data from the Habitats Directive Reports (Mihăilescu et al., 2015b). Only those locations with high spatial accuracy (horizontal error <10 m for both X and Y coordinates) were selected for further analysis and used as input in the Maximum Entropy (MaxEnt) habitat suitability model.

Eight environmental predictor variables were used, grouped into three main categories: geomorphological, climatic, and land use. Geomorphological variables, derived from a 10 m resolution Digital Elevation Model (DEM), included elevation, slope, aspect, and curvature (plan and profile), chosen for their relevance to the species' known preference for humid subalpine terrain. Climatic variables included long-term annual mean temperature and precipitation extracted from the RO-ADAPT platform (1971-2005 baseline). Land use information was provided by the Corine Land Cover Plus (CLC+) dataset, capturing habitat types and anthropogenic disturbance at high spatial resolution.

MaxEnt (v.3.4.1) was run with default parameters, using 75% of the occurrence data for training and 25% for testing. Model performance was evaluated using the Area Under the receiver operating characteristic Curve (AUC), and variable contribution was assessed through percent contribution and permutation importance. The final output consisted of a continuous habitat suitability map with values ranging from 0 (unsuitable) to 1 (highly suitable), interpreted using thresholding and visualized in the geographic information software ArcGIS 8.3.

RESULTS AND DISCUSSIONS

The MaxEnt model simulation for predicting the potential distribution of *T. alpina* subsp. *carpathica* showed an accurate performance, indicated by a high area under the ROC curve (AUC = 0.950)

Potential distribution and suitable area

The habitat suitability model developed using Maxent identifies several core areas with high environmental probability for the presence of *T. alpina* subsp. *carpathica* across the alpine

bioregion of the Romanian Carpathians (Figure 3).

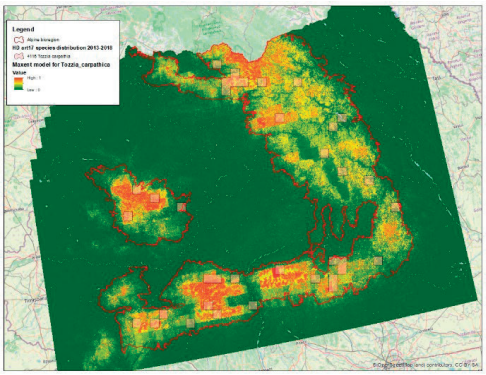


Figure 3. Modelled distribution of *Tozzia alpina* subsp. *carpathica* using Maxent in the Romanian Carpathians

The model predicted high suitability areas in the Eastern Carpathians, particularly in the Rodna and Maramureș Mountains, as well as in the Southern Carpathians, including the Făgăraș and Parâng massifs. Additionally, a significant zone of high suitability is evident in the Apuseni Mountains. These findings are consistent with the current understanding of the species' distribution in Romania. These regions include extensive or clustered patches of suitable habitat, which may support stable metapopulations. An overlay with the 2013–2018 reporting distribution under Article 17 (10 × 10 km grid) confirms the consistency between field records and predicted habitat patches. However, many suitable areas fall outside the currently reported grid cells, indicating a potential underestimation of the species' extent of occurrence. These observations highlight the need for field confirmation and improved monitoring, particularly in areas of fragmented habitat that are predicted to be suitable. Furthermore, the widespread distribution of medium to high suitability patches suggests that *T. alpina* subsp. *carpathica* may be more ecologically flexible than previously assumed but still constrained by microhabitat availability.

Environmental variables

The MaxEnt model revealed that among the eight environmental variables used, land use, elevation and precipitation were the most influential predictors of suitable habitat for *T. alpina* subsp. *carpathica* in the Romanian Carpathians (Figure 4, Table 3).

Table 3. Environmental variables in shaping the species distribution

Environmental variable	Percent contribution	Permutation importance
Land use	45.2	19.5
Elevation	33	42.1
Precipitation	17.7	23.8
Temperature	1.8	0
Exposition	1	4.9
Slope	0.9	6.1
Total curvature	0.3	3.4
Profile curvature	0	0

Land use had the highest contribution (45.2%) to the model, highlighting a strong association between *Tozzia* and specific habitat types. The land use response curve (CLC+ 2018) indicates that *T. alpina* subsp. *carpathica* is primarily associated with areas dominated by coniferous forests (class 2), followed by an association with low-growing woody vegetation (class 5) and permanent herbaceous habitats (class 6). Other land cover categories, including sealed areas, deciduous forests and sparsely vegetated areas, showed minimal influence on the predicted distribution of the species. ow and forest ecotones

Elevation is a key predictor in the habitat suitability model for *T. alpina* subsp. *carpathica*, contributing 33% to the model and showing the highest permutation importance (42.1%). The response curve indicates a lower limit starting at approximately 700–800 m where a significant increase in suitability occurs. Maximum suitability is between 1,200 and 1,800 m, which appears to be the optimal range. After 1,800 m, a significant decrease is observed, suggesting that the species avoids the alpine stage proper (usually >1,800-2,000 m in the Carpathians) and has an ecological preference for montane and subalpine altitudes rather than true alpine habitats.

Precipitation also had a significant influence (17.7% contribution; 23.8% permutation importance), reflecting the species' preference for humid microclimates. Suitable habitats occur predominantly in areas receiving annual rainfall between approximately 600 mm and 1100 mm, with suitability stabilizing at values above this range.

The other chosen variables, such as temperature, slope and curvature, contributed

less (<2%) to the model performance, suggesting that, although they modulate habitat specificity, they are not primary determinants of species distribution.

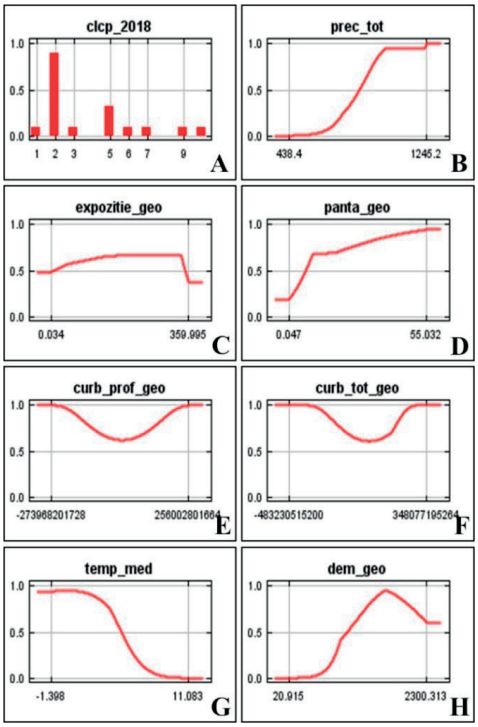


Figure 4. The influence of environmental variables on the predicted habitat suitability for *Tozzia alpina* subsp. *carpathica* in Romanian Carpathians: (A) Land use; (B) Precipitation; (C) Exposition; (D) Slope; (E) Profile curvature; (F) Total curvature; (G) Temperature; (H) Elevation

These findings align with the known ecology of the species, which typically occurs in wet meadows and high herbaceous communities in the montane and subalpine areas of the Carpathians.

The environmental variables that significantly shape the distribution of *T. alpina* subsp. *carpathica*: land use (specific habitats), elevation and precipitation are closely linked to the pressures and threats affecting the species. Given the species' preference for wet habitats with specific hydrological conditions and altitude ranges, it is particularly vulnerable to changes in water regimes, intensive logging activities, changes in land-use management, and the construction of hydropower facilities. In addition, overgrazing and uncontrolled tourist activities, such as motorized vehicles crossing rivers, further degrade the habitat.

Climate change poses another significant threat, as anticipated changes in precipitation patterns - including prolonged drought periods and increased frequency of extreme precipitation events - may lead to surface erosion, changes in soil moisture availability and degradation of suitable microhabitats. Effective conservation of *T. alpina* subsp. *carpathica* thus requires sustainable forestry practices, regulated grazing management and climate adaptive strategies to maintain suitable hydrological and microclimatic conditions within and outside designated protected areas.

List of pressures and threats and conservation measures was updated in 2024 for the period 2019-2024 (EIONET, 2025). During 2007-2023, main conservation measures according with EU code: CB01 - Prevent conversion of (semi-) natural habitats into forests and of (semi)natural forests into intensive forest plantation.

High suitability zones inside and outside the protected areas

The hotspot analysis for the estimated distribution of *T. alpina* subsp. *carpathica* examines the relationship between suitable habitat areas identified by the model, grouping them based on size and connectivity. The results of this analysis are shown in Figure 5.

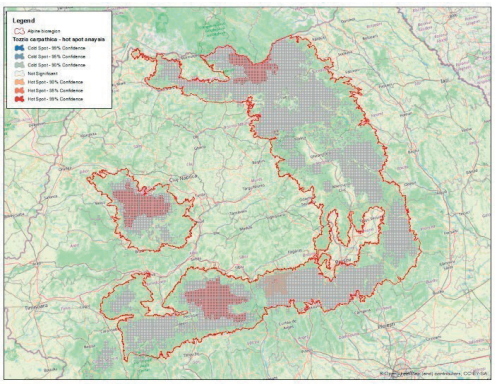


Figure 5. The hotspot analysis for estimated *Tozzia alpina* subsp. *carpathica* areal

A comparative analysis of the two approaches used to estimate the distribution and population of *T. alpina* subsp. *carpathica* (Table 4) highlights the potential advantages of

integrating additional modelling techniques into the Art.17 reporting process. While the Art.17 method provides valuable distribution data at a broad spatial scale (5300 km²), the MaxEnt model suggests a slightly larger potential distribution area (6007 km²) at a significantly finer resolution (0.6 ha versus 100 km²).

Table 4. Comparative analysis on distribution areal of specie *T. alpina* subsp. *carpathica* estimates

Characteristics	MAXENT	Art. 17
Areal distribution	6007 km^2	5300 km^2
Minimum habitat units	0.6 ha	100 km^2
Population size estimates	2.973.017 individuals	300 locations 1x1 km

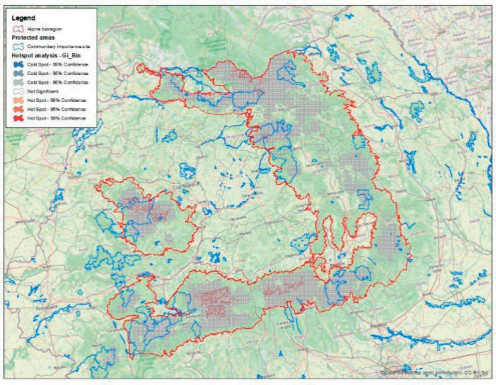


Figure 6. Distribution areal hotspot of species *Tozzia alpina* subsp. *carpathica* in relation with protected areas

By overlapping the distribution area of the suitable habitats with Special Areas of Conservation (SACs) we were able to evaluate the representation proportion for each hotspot class category: "high-confidence" (HC) (>90%), corresponding to extensive areas with favorable conditions for the target species, and the "not significant" category (NS), minor, fragmented patches with limited connectivity potential (Figure 6).

Table 5. Zonation of specific measures on species *Tozzia alpina* subsp. *carpathica* distribution areal

Hotspot class category	Inside SACs	Outside SACs
HC >90	58.87%	41.13%
NS	25.53%	74.47%

The spatial analysis highlights that the distribution of suitable habitats for *T. alpina* subsp. *carpathica* exist both inside and outside SACs. 58.87% of the high-confidence habitats (HC > 90%) are contained in existing SACs, but also a significant proportion (41.13%) remains unprotected.

In contrast, areas designated as "not significant" (NS), predominantly occur outside protected areas (approximately 74%). Only about a quarter (26%) of these NS habitats currently fall within SACs (Table 5).

These results highlight the existence of large and favourable habitat coverage within the SACs.

However, there is a clear need for extended protection or targeted conservation measures to address habitat fragmentation and limited connectivity in areas outside the current SAC boundaries.

CONCLUSIONS

Although the initial hypothesis regarding the underestimated habitat extent was not supported by the MaxEnt analysis, the model makes an important contribution, as it provides higher spatial resolution and precise numerical estimates of potential populations, allowing a more realistic and precise assessment of the conservation status of the species.

Areas identified as “hotspots” can serve as conservation priorities, guiding future monitoring and management measures.

The difference between the reported area (Art.17) and the predicted area (MaxEnt) highlights the need to update monitoring methodologies, including the use of additional predictive models to improve reporting on the status of species of Community interest.

Maintaining the favourable conservation status still depends on the continued application of conservation measures, both for the species and its habitat.

ACKNOWLEDGEMENTS

The data have been obtained in the framework of the Sectorial Operational Programme ENVIRONMENT 2007-2013 (POS no. 130537/10.01.2011) and the Large Infrastructure Operational Programme 2014-

2020 (POIM no. 238/11.03.2019) projects financed by the Ministry of Investments and European Projects. The paper was written in the framework of projects RO1567-IBB01, RO1567-IBB03 and RO1567-IBB04 from Institute of Biology Bucharest of Romanian Academy financed by Romanian Academy.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

STRUCTURAL AND FLORISTIC DIVERSITY OF THE SHRUBLANDS VEGETATION IN THE CĂPĂȚÂNII MOUNTAINS, ROMANIAN CARPATHIANS

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Abstract

The surveys have been carried out in the Căpățânii Mountains, part of the Southern Carpathians, in Romania. In the last 3 decades, the issues of species diversity in shrub vegetation in relation to the evolution, stability and maturity of natural ecosystems have been a priority from an ecological, phytocoenotic and conservation point of view. These shrublands plant communities have some of the greatest plant diversity, characterized by their floristic richness, but which has recently been significantly reduced as a result of a relatively rapid evolution, being under stress from drought, fire, grazing and cutting. In the lower mountain sub-zone, some shrublands plant communities occur, such as: Spiraeo chamaedrifolii-Coryletum avellanae Ujv. 1944. Pinus mugo develops here scrubs between 1,700 and 2,100 m. We found such types of shrublands in the subalpine area, the upper limit of spruces on gentle slopes but also on steep slopes. Alongside these, other shrubby plant communities are also found, which settle on rocky substrates, at the edge of forests or meadows, all of which have an ecological, pedological and conservative role.

Key words*: Căpățânii Mountains, structural diversity, plant communities, habitats, scrublands.*

INTRODUCTION

The establishment and maintenance of shrub vegetation in the mountainous region is of particular importance from an eco-pedogenetic and conservation point of view, especially since these habitats and ecosystems they build are characterized by less favorable environmental conditions.

In the recent years, the shrub vegetation in the mountainous region of the European Mountains has undergone a significant process in terms of physiognomy and floristic structure as well as syndynamics, either due to anthropogenic factors or due to current global climate changes. Among the anthropogenic factors that have had a negative effect on shrub vegetation, we can mention the extensive grazing of animals in close correlation with the fires made in order to increase the grazing area in the mountainous region, waste resulting from tourism or construction, mining and material extraction. This paper presents studies on the structure and dynamics of shrub vegetation in the Capățânii Mountains, an integral part of the Romanian Carpathians. The shrub vegetation in the Romanian Carpathians is very diverse, having a complex structure and often creating natural habitats of community interest or priority within protected natural areas.

MATERIALS AND METHODS

Study area

The territory under study refers to the the mountainous level pertains to the central-European province, the Carpathian sub-province, the land of the Meridional Carpathians, the Western District (Godeanu-Parâng). From an orographic point of view, the Căpăţânii Mountains present a high, main, bare ridge (crest) oriented in the east-west direction, from which there are numerous ramifications, to the north, as well as to the south, which have the shape of edges and crests and which locals call “plaiuri”.

These mountains stand out by their deeply fragmented and well developed massive relief (Popescu, 1968; Niculescu, 2006). The most important crests and ridges of these mountains, belonging to the, are the following: Ursu Peak (2124 m), Nedeia (2130 m), Negovanu (2064 m), Beleoaia (2109 m), Căpăţâna Peak (2113 m), Govora Peak (1958 m), Zmeuretu Mountain (1979 m), Balota Peak (2094 m), Coşana Peak (2080 m), Corșor Peak (2001 m),

Ionașcu Peak (1979 m), Turcinu Peak (1963 m), Priota Peak (1954 m), Bogdanu Peak (1881 m), Gera Peak (1856 m), Târnovu Mare Peak (1846 m), Văleanu Peak (1847 m), Funicelul (1820 m) Mountain, Piatra Roşie Mountain (1820 m), Roman's Peak (1810 m), Dârjala Mountain (1900 m), Vioreanu Peak (1866 m), Vânturarița Peak (1885 m), Buila Peak (1847), Petriceaua Mountain (1650 m), Căşăriei Mountain (1721 m). In the highest part of Căpățâna Mountains there is still some evidence of glacial activity.

Therefore, a well defined cove which has the shape of a circle can be noticed on the Eastern side of Ursu Peak (Popescu, 1968; Niculescu, 2006). The Capatanii Mountains have three secondary ridges: Buila-Vânturarița, Piatra Târnovului and Foarfeca Nărățului. In the Căpățânii Mountains there are important hydrographic basins, Olteț, Cerna, Luncavăț, Bistița, Horezu, Repedea, Govora, Cheia, Olănești. We can also mention important gorges that are found in the Căpățânii Mountains: Olteț, Bistriței, Costești, Cheia, Albeștenilor Gorges. Several protected areas are found in the studied territory: ROSAC0128 Nordul Gorjului de Est, Buila-Vânturaița National Park, Cozia National Park (eastern part of the Căpățânii Mountains, called Narățu) and ROSAC0239 Tarnovu Mare-Latoriței.

The climate of the studied territory is the result of complex interactions between solar radiation, the particularities of the relief and of the atmospheric circulation, characteristic to this area.

The relief acts upon the meteorological elements by its altitudinal development, by the orientation and the inclination of the slopes, by the configuration of the main relief units, determining the climatic disposal of the territory in floors and a multitude of topo-climates. On the investigated territory, one can notice the following climatic floors: mountainous and subalpine (Niculescu, 2006).

Methods

To identify the species and the inter-taxa, we looked into: *Romanian Flora*, vol. I-XII (Săvulescu coord., 1952-1976); *Flora Europaea*, vol. I-V (Tutin et al., 1964-1980); *Flora Italica*, vol. I-II, by P. Zangheri (1976); *The Romania illustrated Flora - Pteridophyta et Spermatophyta*, by V. Ciocârlan (2000, 2009); *Spontaneous and cultivated wooden flora in Romania*, vol. I-II, by V. Zanoschi, I. Sârbu et Angela Toniuc (1996, 2000); Alpin *Flora*, Vol.I, II, III by D. Aeschimann et al. (2004). For the study of the vegetal carpet in the Căpățânii Mountains, we have used methods of phyto-sociologic research characteristic to the Central European phyto-sociologic School, which was based on the principles and methods elaborated by J. Braun-Blanquet (1928) adapted by A. Borza (1934) Borza and Boșcaiu (1965) to the particularities of our country's vegetation (Niculescu, 2006).

The size of the sample areas was: 25-100 m², characteristic of shrub vegetation. The synthetic tables of the described vegetal associations contain information referring to: number of surveys, altitude (m.s.m), exposure, inclination (in grades), the completion of the crowning (where it is the case), vegetation level of covering (%) and the analyzed surface (m²). The vertical arrangement of the phytosociological tables was done according to the coenotaxonomic criterion. The quantitative assessment of the participation of every species to the vegetal association was performed with the help of the abundance-dominance index, according to the Braun-Blanquet scale. We have noted the constancy of the species (K) in the case of the plant communities. As for the classification of the plant communities, we have used synthesis papers on the Romanian vegetation, elaborated by G. Coldea (1986, 1991, 1997, 2015), V. Sanda et al. (1997) as well as other recent belonging to the authors: E. Oberdorfer (1992, 1994), Mucina et al. (1993, 1997, 2016), Rodwell et al. (2002).

RESULTS AND DISCUSSIONS

The shrub vegetation in the Căpățânii Mountains is extremely rich and diverse due to the very varied ecological, geomorphological and climatic conditions in this territory. The coenotaxonomic overview was carried out based on our own research carried out between 1997-2024, as well as data from specialized literature regarding this area.

The shrubby vegetation in this territory is found both in the mountain and in the subalpine levels, either on rocky substrate, on crystalline schists

or limestone, either at the edge of forests, or on the banks of streams, in humid and cool or shady valleys, or interrupting the continuity of mountain or subalpine meadows.

The structure of phytocoenoses of plant communities varies depending on eco-pedo-climatic conditions in close correlation with the intensity and types of anthropogenic pressures as well as the implementation of appropriate management for each type of vegetation. Following the studies carried out, but also based on the specialized literature, a fairly large number of shrubby plant communities were identified in the Căpățânii Mountains.

These are classified into many categories:

I. ***Shrubby plant communities established in forest clearings***

These plant communities have a high intensity of synanamics, they are also established on mobile debris on sloping slopes, but also on flat terrain, in the forest floor of beech, spruce, or mixtures of beech with fir or beech with fir and spruce. The following plant communities fall into this category:

1. The plant community *Rubetum idaei*

Gams 1927 (Syntax. Syn.: *Fragario vesca-Rubetum idaei* (Pfeiffer 1936) Siss. 1936) has a wide distribution in the Căpățânii Mountains, occurring in all the hydrographic basins: Olteț, Luncavăț (Niculescu, 2006), Olănești, Cheia, Bistrița-Vîlcii (Popescu, 1974), Horezu, Govora, Cerna de Olteț (Răduțoiu, 2008), Stan Valley, Suhăioasa, Cîrtstoiu, Sașa, Cioroiu, Plescioara, Mesteacanul, Niculii, Sturișori, Grotului, Izvorul Mare. It is frequently spread from the mountain to the subalpine level, forming a serial vegetation on the lands where forestry exploitations have been made, but also at the edge of the forests, in forest clearings, where there are favorable ecological conditions, based on a well-individualized coenotic nucleus in which the edificator species is *Rubus idaus* which has a high abundance-dominance (3-5) and a high constancy.

This plant community presents pedogenetic, alimentary, phytopharmaceutical, but also conservative importance.

2. *Spiraeo-Coryletum* Ujv. 1944

(Syntax. Syn.: *Calamagrostio arundinaceae - Spiraeetum ulmifoliae* Resmeriță et Csürös 1966) is a plant community of succession to acidophilous beech forests. The germander meadowsweet and common hazel phytocoenoses are spread in the mountain floor, at altitudes between 750-1550 m on rocky slopes, with different exposures, whose inclination is between 20° and 70°. In the Căpățânii Mountains this plant community is found in the Valleys: Luncavăț, Curpenilor, Polovrăgeni, Suhăioasa, Olănești, Otăsău, Govora, Cheia, Bistrița-Vîlcii, Olteț, Costești, Stan's Valley and also on the Culmea Tribunalului. It is a succession palnt community towards acidophilic beech trees in southern Romania.

The phytocoenoses of this plant community are characterized by a well-established shrub layer, in which, alongside the edifying species, one also encounters: *Rosa pendulina, Lonicera nigra, Rubus idaeus, Sambucus racemosa*. In the grassy layer, some species have a high dominance abundance (3-4) and high constancy: *Calamagrostis arundinacea, Luzula luzuloides, Veronica urticifolia, Poa nemoralis, Valeriana tripteris Saxifraga cuneifolia, Aplenium trichomanes, Polypodium vulgare, Selaginella helvetica*.

3. *Calamagrostio arundinaceae-Sambucetum racemosae* Resmeriță (1970) 1986 (Syntax. Syn.: *Sambucetum racemosae* (Noirf. 1940) Oberd. 1973; *Sambuco-Calamagrostietum arundinaceae* Resmeriță (1970) palnt community edifying by red elderberry and bunch grass, is found in the forest clearings in the beech and fir-beech areas. This plant community is found sporadically on soils rich in organic substances, on semi-shady slopes, with an inclination between 15-35°.

In the researched territory, phytocoenoses of this association were identified in the upper basin of the Luncavăț river, in the valleys of the streams Râmești, Blajului, Costești, Stan, Văleanu, Suhăioasa, Culmea cu Larice and also in the upper basin of the Govora and Olanești rivers, at altitudes between 900-1400 m.

In the coenotic structure of this plant community, species characteristic of the *Sambuco-Salicion* alliance of the *Querco-Fagetea* class, of the *Fagetalia* order, participate with high constancy.

In the floristic composition, tree shoots from the initial plant community are also found (Niculescu, 2006). The vegetation coverage is between 80-100%.

II. ***Shrubby plant communities established on the banks of streams, mountain springs or on moist and shady valleys in the mountain floor***

This type of shrubby vegetation is also well represented in the Căpățânii Mountains. Thus, the following plant communities are very widespread in the researched territory:

1. The plant community *Salicetum purpureae* (Soó 1934 n.n.) Wendelbg-Zelinky 1952 is frequently found in the form of small strips or clumps on the banks of the ravines in the lower montane undergrowth of the Căpățânii Mountains.

The phytocoenoses of this plant community develop on moist soils, and have a shrubby state dominated by the edifying species *Salix purpurea*, alongside which *Salix fragilis* and *Salix alba* are also found with high constancy. Such phytocenoses are found in the Luncavățului Valley, in Vaideeni and Horezu, in the Bistriței-Vâlcii Valley, in Costești, Bărbătești and Pietrari de Sus, in the Cernei de Olteț Valley, in Marița, in the Oltețului Valley in Polovragi, in the Govorei Valley, in Stoienești and in the valleys of the Cheia and Olanești streams. This plant community is characterized by a heterogeneous floristic composition of the grassy layer, in which some species belonging to the *Salicion* and *Alno - Ulmion* alliances, the *Salicetalia purpureae* order and the *Molinio-Arrhenatheretea* class have a high abundance-dominance and a high constancy.

From a syndinamically point of view, the phytocoenoses of the described plant community evolve towards mountain alder forests, but under the pressure of the anthropogenic factor they undergo a process of weeding and ruderalization, which may lead to the development of vegetation characteristic of grasslands from the *Agropyro-Rumicion* alliance.

2. *Salicetum cinereae* Zólyomi 1931 (Syntax. Syn.: *Salici cinereae-Sphagnetum recurvi* (Zóly 1934) Soó 1955); *Calamagrostids lanceolata* Dihoru 1965) plant community edifying by *Salix cinerea* and *Calamagrostis canescens* which settles on alluvial, humid soils with an acidic pH. It is a less widespread woody plant community in the Căpățânii Mountains, occurring in the middle and upper mountain undergrowth, in the stream basins: Luncavăț, Curpenilor, Sașa, Cioroiu, Niculii, Sturișori, Izvorul Mare, Cheia, Olteț, Stan Valley and on the Culmea cu Larice (Niculescu, 2006). This plant community is also frequently found on the shady and steep valleys of the Buila-Vânturarița Massif. The phytocenoses of the Căpățânii Mountains have a well-individualized cenotic core, along with the edifying species, numerous species characteristic of the *Salicion cinereae* alliance, *Alnion incanae*, but also of the *Alnetalia* glutinosae class are found. The shrub cover is between 70-90%, the grassy layer is poorly developed, *Sphagnum recurvum* being absent in some phytocoenoses or having a low abundance-dominance.

3. The plant community *Salicetum albae* Issler is not widely distributed in the mountain area. Phytocoenoses of this plant community are found in the Luncavățului Valley, the Râmești Valley, in the localities of Ifrimești and Râmești, but also in the Olănești Valley. Some of the analyzed phytocoenoses are presented in the form of narrow strips. These phytocoenoses are dominated by *Salix alba* and achieve a coverage of 60-85%, with a heterogeneous, well-developed grassy layer formed mainly by species belonging to the *Salicetea purpurea* class and which participate together with the edifying species in the fixation of the alluvial substrate, of the pebbles. With a relatively low constancy, species from the *Molinia-Arrhenatheretea* class are also found, migrated from the contact plant communities. As a result of the high level of soil humidity, a series of meso-hygrophilous and even hygrophilous species also participate in the floristic composition of these phytocenoses.

4. *Salici purpureae-Myricarietum* Moor 1958 (Figures 1, 2) is a rarely encountered plant community in the Căpățânii Mountains.

The phytocoenoses of this plant community are found on the floodplain pebbles of the mountain meadows, on alluvial soils in several locations in the upper basin of the Luncavăț at an altitude of 750 m (Niculescu, 2006). In the Căpățânii Mountains, such phytocenoses were also found fragmentarily in the Costești Valley and Izvorul Mare (on the northern slope of these mountains). The edifying species of this association are *Salix purpurea* and *Myricaria germanica*. In the floristic composition, a series of characteristic species of Salicion-elaeagni and *Salicetalia purpureae* are found, such as: *Salix alba, S.*

fragilis, Mentha longifolia, Lysimachia numularia, Sapponaria officinalis (Nuță et Niculescu, 2019).



Figure 1. *Salici purpureae-Myricarietum* Moor 1958 plant community in the upper basin of the Luncavăț river Luncavățului

Also present are numerous species belonging to the *Alno-Ulmion* alliance, including *Alnus incana, Alnus glutinosa, Stellaria nemorum, Impatiens noli-tangere, Aegopodium podagraria*. The presence of species from this alliance indicates that this group is evolving towards phytocoenoses built by *Alnus incana* (Niculescu, 2006; 2015).



Figure 2. *Salici purpureae-Myricarietum* Moor 1958 plant community in the Costești Valley

5. *Saliceto silesiacae-Alnetum alnobetulae* Colic et al. 1962 (Syntax. Syn.: *Alnetum viridis* auct roman) plant community includes the mountain alder thickets that are found along shady valleys, on steep slopes, in rocky and humid places. In the Capățânii Mountains, this plant community is widespread in the upper basins of most valleys in the mountain and subalpine levels, often at the border of spruce forests or mixed forests with fir and beech. The plant community built by Silesian willow and Green alder was identified in the Luncavăț Basin (Niculescu, 2006; 2016), Cernei de Olteț (Răduțoiu, 2008), Bistriței-Vîlcii (Popescu, 1974). Following the research we conducted in the Căpățânii Mountains, this plant community was also identified in the Olteț, Govorei, Olănești, Cheia, Suhăioasa, Izvorul Mare basins, on the shady and humid valleys, especially with western and northern exposure, in the Buila - Vânturarița Massif. Although this association presents the most extensive development in the subalpine level, in the upper Luncavăț basin, such phytocoenoses were also frequently encountered in the mountain level. Thus, in the upper Luncavăț basin, this plant community has a wide amplitude, meeting in the Curpenilor Valley, in the area of Lacul Ursu, Stâna Balota and Culmea cu Larice, at altitudes between 900-1800 m. They settle on slopes with an inclination between 10-35° and a generally western exposure and have a predominantly mesophilic, microthermal and acido-neutrophilic character (Niculescu, 2006). From the Cerna de Olteț stream basin, it was collected from the Recea Valley, the Țiganului River, the Strâmtori Hill and the Mariţa River Valley (Răduțoiu, 2008).

The coenotic nucleus includes, in addition to the two edifying species, a series of weed species belonging to the *Adenostylion alliariae* alliance and the *Betulo-Adenostyletea* class: *Doronicum austriacum, Leucanthemum waldsteini, Senecio germanicus, Valeriana tripteris, Ranunculus platanifolius, Viola biflora*. Considering that the phytocoenoses of this plant community are located in the immediate vicinity of spruce forests, a series of species from neighboring spruces are also present in the floristic structure, such as: *Picea abies, Vaccinium myrtilus, Rubus hirtus, Viola reinchenbachiana, Veronica urticifolia.* This plant community is of particular importance in soil fixation, and intervenes in regulating water runoff from melting snow (Niculescu, 2006).

III. ***Mountain deciduous shrubs***

Within this category, we have included two less researched plant communities in Romania and which were cited from few places in the country,

from the Căpățânii Mountains being cited only from the upper Luncavăț basin.

1. *Rubo plicatae-Prunetum spinosa* Web. 1974 em. Oberd. 1992 (Syntax. Syn.: *Rubo plicatae-Coryletum avellanae* Oberd. 1957) plant community has been little researched in our country, being cited only from the Crişului Gorge. It was cited for the first time from Oltenia, from the upper Luncavăț basin (Niculescu, 2006). Thus, in the studied territory the plant community is found in the lower mountain substratum, preferring especially humid places, at the edge of forests. Phytocoenoses of this association were analyzed on V. Râmeşti, in the localities: Ifrimeşti, Râmeşti and Bălăneşti (Niculescu, 2006). From an ecological point of view the analyzed phytocoenoses have a strong mesophilic, micromesothermic and euryionic character.

From a structural point of view, alongside the edifying species - *Rubus plicatus, Prunus spinosa* and *Corylus avellana*, the following species are also found with high abundance-dominance and high constancy: *Ligustrum vulgare, Euonymus europaeus, E. verrucosa, Cornus sanguinea, Clematis vitalba, Crataegus monogyna, Sambucus nigra, Urtica dioica, Brachypodium sylvaticum, Veronica chamaedrys, Geum urbanum, Campanula rapunculoides, Astragalus glycyphyllos, Prunella vulgaris, Euphorbia amygdaloides.*

2. The plant community *Pruno spinosae-Ligustretum vulgare* Tx. 1952 (Syntax. Syn.: *Euonymo-Prunetum spinosae* (Hueck 1931) Tx. 1952 em. Pass. et Hofm. 1968, *Pteridio-Crataegetum monogynae* Raţiu et Gergely 1970, *Carpino-Prunetum* Tx. 1952, *Rosetum rhamnosum* sensu Borza 1959) (Figure 3) like the previous plant community, it represents a less researched coenotaxonomic group of shrubs in our country. This plant community is edified up by *Ligustrum vulgare* and *Prunus spinosa* and was cited for the first time from Oltenia, from the Căpățânii Mountains (Niculescu, 2006; 2020). Following the studies carried out, the floristic structure and ecology of the phytocenoses of this plant community on the Râmeşti Valley in the Râmeşti and Bălăneşti localities were described. The phytocenoses are established in humid places, on nutrient-rich soils, on the edges of roads or forests, on slopes with an inclination between 5-30° and generally facing west.



Figure 3. *Pruno spinosae-Ligustretum vulgare* Tx. 1952 plant community in the Râmeşti Valley

The coenotic nucleus is well individualized, characteristic of marginal forest vegetation and includes a series of species belonging to the *Crataego-Prunetea* class and the *Prunetalia spinosae* order: *Euonymus europaea, Cornus sanguinea, Rosa canina, Clematis vitalba, Crataegus monogyna, Clinopodium vulgare*, *Glechoma hederacea, Veronica chamaedry*, *Origanum vulgare*. At the same time, the species that differentiate the *Carpino-Prunion* alliance - *Dactylis glomerata, Geum urbanum, Urtica dioica* frequently appear in some phytocoenoses. Sometimes the phytocoenoses of this plant community form the hedges at the border of some localities in the upper basin of the Luncavăț river and the Bistrița-Vîlcea Valley, on heat-loving and richly alkaline soils. The plant community is of particular importance from a pedogenetic and conservation point of view.

IV. ***Low shrubs from the montane and subalpine levels***

This group of plant communities groups together low bushes from the Căpățânii Mountains in which camephytes and nanophanerophytes species predominate and have a heterogeneous floristic structure because in this ecotonal area there is the influence of several different coenotaxonomic vegetation units.

1. The plant community *Campanulo abietinae - Juniperetum alpinae* (Buia et al. 1962) Boşcaiu 1971(Syntax. Syn.: *Juniperetum nanae* Soó 1928, *Juniperetum sibiricae* Raţiu 1965) is dominated by dwarf juniper, and is

frequently found in the upper montane substratum and the subalpine stage, on slightly inclined and semi-sunny slopes. This plant community has a wide distribution in almost all basins of the Căpățânii Mountains: the upper basin of the Luncavăț river, (Șaua Funicelul, Mountains Piatra Roșie, Ursulețul, Dârjala, Căşăriei, La Colțul Marginii, under Balota and Căpățâna Peaks), Oltețului basin (Curmătura Oltețului, Boul Peak), Bistrița-Vîlcii basin (Văleanu, Zonoaga and Zmeurăt Peaks, Curmătura Rodeanu) as well as on the northern slope of the Căpățânii Mountains (Piatra Târnovului, Vârful lui Stan, Culmea Durducului), the Olănești basin (Folea Mountain) and the Cheia basin (Oale Mountain). From the point of view of the coenotic structure, along with the edifying species *Juniperus communis* ssp. *alpina* and *Campanula patula* ssp. *abietina*, the following species participate with high constancy: *Vaccinium myrtillus, V. vitis-idaea, Chamaenerion angustifolium, Rubus idaeus, Luzula luzuloides, Senecio ovatus, Bruckenthalia spiculifolia, Rhododendron myrtifolium, Homogyne alpina, Deschampsia flexuosa, Cetraria islandica*.

The phytocoenoses of this plant group are of particular importance from a conservative but also pedogenetic point of view, and in unfavorable ecological conditions they can evolve into *Nardus stricta* or *Festuca nigrescens* meadows.

2. *Campanulo abietinae-Vaccinietum myrtilli* (Buia et al. 1962) Boşcaiu 1971 (Syntax. Syn.: *Vaccinietum myrtilli* Buia et al. 1962, *Junceto trifidi-Vaccinietum* Resmeriță 1976) plant community (Figure 4) is widely distributed in the Căpățânii Mountains, occurring in the montane and subalpine layers. The plant community edified up by the blueberry is found at the upper limit of the spruces, on sunny and semi-sunny slopes in all hydrographic basins of the Căpățânii Mountains, including the Buila-Vânturarița Massif, which is an integral part of these mountains. In the upper basin of the Luncavăț river, phytocoenoses of this plant community occupy large areas in the Șaua Funicelul, on Piatra Roșie Mountain, Dârjala Mountain, Ursulețul Mountain, Blajului Valley and Izvorul Sec. These thickets have developed greatly on the cleared areas of the border forests. In the Olteț basin, the plant community is found in Curmătura Oltețului, on the Boul, Turcinu and Negoveanu Peaks, on the valleys of the Datcului, Curmăturii, Urliesul, Dracului streams. In the Cerna de Olteț basin, this group of species occupies large areas on Mount Pleșa, Zăvidanu, Corșor, Senănările, Milescu. From this basin, the plant community was cited from the Buciumu, Corșoru and Beleoaia Peaks (Răduțoiu, 2008). In the floristic composition of these phytocoenoses, along with *Vaccinium myrtillus* and *Campanula patula* ssp. *abietina*, the following species are also frequently found: *Vacinium vitis-idaea, Deschampsia flexuosa, Bruckenthalia spiculifolia, Rhododendron myrtifolium, Homogyne alpina, Cetraria islandica*.

From a syndinamically point of view, we can say that blueberry phytocoenoses settle in spruce forest clearings and can evolve into nardete. They are of particular importance from a conservative, economic and eco-pedogenetic point of view.



Figure 4. *Campanulo abietinae-Vaccinietum myrtilli* (Buia et al. 1962) Boşcaiu 1971 in the Căpățânii Mountains

3. *Junipero alpinae-Bruckenthalietum spiculifoliae* Horv. 1936 (Syntax. Syn.: *Juniperetum intermediae* Nyár. 1956 n.n., *Bruckenthalietum spiculifoliae* Buia et al. 1962 p.p., ass. with *Bruckenthalia spiculifolia* and *Antennaria dioica* Şerbănescu 1961, ass. with *Nardus stricta* and *Bruckenthalia spiculifolia*) plant community it is less widespread in the Căpățânii Mountains, the upper basin of the Luncavăț river, Bistrița-Vîlcii, Otăsău, Olteț, Repedea, Cărpănoasa, Horezu, Bacea, Roșu and Funicelu Valleys. Important areas of these plant communities are also found in the Buila-Vânturarița Massif, especially on Vânturarița

Mare, Cumătura Builei and Curmătura Oale where they have a very good development, *Bruckenthalia spiculifolia* is almost absent and there is a strong intercalation of the community and the steep built-up plant community of dwarf mountain pine. In the upper basin of Luncavăț river, the phytocoenoses of this plant community are widely spread, meeting in the following resorts: Piatra Roşie, Balota, Dârjala, Căşăriei Mountains, Şaua Funicelul, Ursu and Căpăţâna Sheepfolds (Figure 5) (Niculescu, 2006).



Figure 5. *Junipero alpinae-Bruckenthalietum spiculifoliae* Horv. 1936 on the Balota Mountain

From the Cerna de Olteț basin this plant community was classified as *Antenario dioicae-Bruckenthalietum spiculifoliae* I. Şerbănescu 1961 from the Corşoru, Beleoaia, Buciumu, La Nedei Peaks at altitudes between 1600-1850 m (Răduțoiu, 2008).

The phytocoenoses of these plant communities are edified by *Juniperus communis* ssp. *alpina* and *Bruckenthalia spiculifoila* and have a general coverage between 80-100%. In the floristic composition of these phytocoenoses, in a fairly large number of cormophytes species, of which a high constancy or in the following species: *Campanula patula* ssp. *abietina*, *Vaccinium myrtillus, V. vitis-idaea, Potentilla aurea ssp. chrysocrapeda, Homogyne alpina, Nardus stricta, Descampsia flexuosa, Pulsatilla alba, Geum montanum, Genista tinctoria* ssp. *oligosperma, Antennaria dioica*. From a synanthropic point of view, these phytocoenoses are similar to the *Nardus stricta* meadows and this plant community plays a very important role from a conservative and eco-pedogenetic point of view.

4. *Cetrario islandicae-Loiseleurietum procumbentis* Br.-Bl. et. al 1939 (Syntax. Syn.: *Loiseleurietum procumbentis* Rűb. 1931) plant community (Figure 6) is edified by the species popularly called alpine azalea and true iceland lichen. *Loiseleuria procumbens* (L.) Desv. (Syn. *Kalmia procumbens* (L.) Gift & Kron & P. F. Stevens ex Galasso, Banfi & F. Conti; *Azalea procumbens* L. *Chamaecistus procumbens* (L.) Kuntze) in association with *Cetraria islandica* (L.) Ach. it develops on the ridges and gentle slopes of high peaks in the subalpine zone, exposed to cold winds, at altitudes between 1900 m and 2050 m (Niculescu, 2003; 2006). The largest areas of this plant community were found in the upper basin of Luncavăț river, on the Balota, Căpăţâna, Ursu Peaks and Curmătura Funicelul (Niculescu, 2003; 2006).

In the Cerna de Olteț basin, such phytocoenoses were identified on small areas on two mountain peaks: Buciumu and Corşoru (Răduțoiu, 2008). The plant community was also cited from the Bistrița-Vâlcii basin, where it also occupies areas on high, windy plateaus with skeletal, superficial soils, poor in nutrients.

From an ecological point of view, these phytocoenoses have a strong mesophyle, microthermic, criophyle and acidophyle character. The coenotic nucleus is dominated by this dwarf azalea, with creeping stems. The floristic structure of the phytocoenoses includes a small number of species, due to the harsh ecological conditions, of which, in addition to the species characteristic of the alliance, we can mention: *Phyteuma confusum, Campanula alpina, Juncus trifidus, Primula minima, Agrostis rupestris, Festuca airoides, Hieracium alpinum* etc. The degree of vegetation coverage is between 75% and 100%.



Figure 6. *Cetrario islandicae-Loiseleurietum procumbentis* Br.-Bl. et. al 1939 on the Căpăţâna Peak

5. The plant community *Cetrario islandicae-Vaccinietum gaultherioidis* Boşcaiu 1971 prefers the strongly windy ridges and plateaus on the tops of the mountains. This plant community is found in the following hydrographic basins: Luncavăţ, Olteţ, Bistriţa-Vîlcii, Cerenei de Olteţ, Olaneşti, Cheia, Roşu, Repedea, Huluzu, Funicelu, Groşeţu, Cărpănoasa, Grotului. In the upper basin of Luncavăţ, these low bushes are found on Mount Piatra Roşie, Căpăţâna, on the Balota, Căpăţâna, Coşana and Ursu Peaks, at altitudes between 1810 m and 2100 m, growing on skeletal, strongly podzolic soils (Niculescu, 2003; 2006). In the Cerna de Olteţ basin, this association is present on the ridges of three mountain peaks: Corşoru, La Nedei and Buciumu, at variable altitudes between 1800 and 2000 m (Răduţoiu, 2008). The phytocoenoses marked by good development and a well-individualized species nucleus are also found in the Buila-Vânturariţa Massif.

From an ecological point of view, the phytocoenoses of this plant community are characterized by the predominance of mesophyle, microthermic and acidophyle species. The floristic structure of these phytocenoses includes, along with the two edifying species, a series of subalpine and alpine species: *Loiseleuria procumbens, Thamnolia vermicularis, Agrostis rupestris, Pulsatilla alba, Homogyne alpina, Carex curvula, Hieracium alpinum, Campanula alpina* etc. This plant community plays a very important role in soil protection. This plant community is also of particular importance from a conservation point of view, also edifying the 4060 Alpine and Boreal heaths natural habitat.

6. *Rhododendendro myrtifolii-Vaccinietum* Borza (1955) 1959 em. Boşcaiu 1971 (Figure 7) is a plant community edyfid by carpathian rhododendron and bilbertrry it is quite widespread in the Căpăţânii Mountains, having a particularly important role from a floristic, ecological and especially conservative point of view. In the upper basin of the Luncavăţ river, such thickets occupy important areas and are spread in the subalpine layer, occupying relatively large areas on the Balota, Căpăţâna, Coşana, Ursu Peaks, at Izvorul Sec and in the Funicelul Saddle. Also important areas of this plant community are found in the Bistriţa Vîlcii basin (Popescu, 1974), Oleţului, on Piatra Târnovului, on the Nedei Peaks, Negoveanul, Turcinu, Boul, on the Roşu, Funicelu, Groşetu, Cârpănoasa, Căprăreasa streams. The phytocoenoses of this plant community have a distinct floristic structure, characteristic of the slopes of the subalpine layer, with humic-silicate, superficial, nutrient-poor and highly acidic soils. Among the species that participate in the floristic composition, the following are frequently encountered: *Vaccinium gaultherioides, V. myrtillus, Juniperus communis* ssp. *alpina, Homogyne alpina, Agrostis rupestris, Juncus trifidus, Potentilla aurea* ssp. *Chrysocraspeda, Hypericum richer* ssp. *grisebachii, Campanula serrata.* In the upper basin of the Luncavăţ river, these plant communities have a secondary character. Those established on the Căpăţâna and Ursu Peaks can evolve into meadows of *Festuca airoides* or *Juncus trifidus* but also into the plant community edified up by *Pinus mugo*, thus contributing to the conservation of the natural heritage in the area. This group of plants has a special importance from a conservative, phytopharmaceutical, eco-pedological, economic point of view.



Figure 7. *Rhododendendro myrtifolii-Vaccinietum* Borza (1955) 1959 em. Boşcaiu 1971 in the Şaua Funicelu

7. *Juniperetum sabinae* Csűrös 1958 is a plant community found on sunny limestone cliffs, on very steep slopes of the Vânturaiţa-Buila Massif, on the Costeşti Gorges, the Cheia Valleys and the Olăneşti Valleys. The phytocenoses that build this plant community have the appearance of elongated bushes with a maximum shrub layer coverage of 85%. In the floristic structure of the phytocenoses, a small

number of cormophyte species are found, characteristic of the rocky substrate of the Southern Carpathians, many of which belong to the *Sesleritalia* class. This plant community is of great importance from a conservation point of view. The edifying species has a special role against the erosion of the substrate on which it develops. Also, in the floristic composition, many rare and endemic species are found.

V. ***Plant communities built by dwarf mountain pine***

1. *Rhododendro myrtifolii-Pinetum mugo* Coldea 1985 (Syntax. Syn.: *Pinetum mugo carpaticum* auct. roman) is a plant community spread in the subalpine storey of the Căpățânii Mountains (Figure 8), at the upper limit of spruces, on gentle slopes but also on steep slopes, occupying the largest areas in the upper basin of the Luncavăț river. *Pinus mugo* forms thick and difficult to cross underwoods, at an altitude between 1,650 and 1,950 m.

In the upper basin of the Luncavăț river we find it under Balota Peak, Căpățâna Peak, Șaua Funicelu, Izvorul Sec (Niculescu, 2003; 2006).

The shrubby phytocoenoses built up by *Pinus mugo* have a more restricted area in the researched territory. They are located at variable altitudes, between 1700-1900 m on 2 mountain peaks: La Nedei and Beleoaia (Răduțoiu, 2008).



Figure 8. *Rhododendro myrtifolii-Pinetum mugo* Coldea 1985 in the Căpățânii Peak

In the Căpățânii Mountains, this plant community occupies relatively large areas and in the Bistrița-Vîlcii basin (Popescu, 1974). At the same time, it is found on Tîrnovul Mare, Piatra Târnovului, in the Buila-Vânturarița Massif (on Vânturarița Mare, Stogu, Creasta Builei, Curmătura Builei, Curmătura Oale) especially among the limestone rocks. In the upper basin of the Luncavăț river, phytocoenoses edified by *Pinus mugo* are also found in the upper mountain floor, descending under the spruce forests, up to an altitude of approx. 1400 m.

This was observed in the summer of 2003, in the place called Izvorul Sec (Niculescu, 2006). Many years ago, during the communist period, there was a desire to intensify pastoral activity in the mountain area, so an increasingly larger pasture area was needed. In order to increase this area for expansion, intense actions of burning and deforestation of junipers in the Căpățânii Mountains took place.

This led to the destruction or considerable reduction of the areas of the juniper habitat in this area, but also in other mountainous areas in Romania. The clearing of the junipers in the Funicelu Saddle and at Izvorul Sec was done in the form of transverse strips (Figure 9), which explains their current appearance (Niculescu, 2006).



Figure 9. *Pinus mugo* phytocoenoses in the form of bands in the Șaua Funicelu

In the composition of the shrub layer, along with the dominant species *Pinus mugo*, such as: *Sorbus aucuparia, Juniperus communis* ssp. *alpina, Picea abies* also participate. In the grass layer, the following species are frequently found: *Vaccinium myrtillus, V. vitis-idaea, Soldanella hungarica ssp. major, Deschampsia flexuosa, Homogyne alpina, Bruckenthalia spiculifolia.*

Following deforestation, these phytocoenoses can evolve into meadows of *Nardus stricta* or *Festuca nigrescens*. In some stations established following the burning of junipers, the establishment of phytocenoses of mountain peony with blueberry was observed.

In the Căpățânii Mountains, this group of plants is interspersed especially with phytocoenoses of *Juniperus communis* ssp. *alpina* (Figure 10).



Figure 10. Intercalation of *Juniperus communis* ssp. *alpina* and *Pinus mugo* phytocoenoses on Căpățâna Peak

From a conservation point of view, most of the shrubby plant communities described in the Căpățânii Mountains constitute Natura 2000 sites of community or priority interest as follows:

1. 4060 - Alpine and Boreal heaths (CLAS. PAL.: 31.4) habitat (Gafta and Mountford, coord., 2008) in the Căpățânii Mountains is edified by the following plant communities:
- *Cetrario-Loiseleurietum procumbentis* Br.-Bl. et al. 1939;
- *Rhododendro myrtifolii-Vaccinietum* Borza (1955) 1959 em. Boşcaiu 1971;
- *Junipero-Bruckenthalietum* Horvat 1936;
- *Cetrario- Vaccinietum gaultherioidis* Boşcaiu 1971;
- *Campanulo abietinae-Vaccinietum* (Buia et al. 1962) Boşcaiu 1971;
- *Campanulo abietinae-Juniperetum* Simon 1966;
- *Juniperetum sabinae* Csűrös 1958;

2. 4070* Bushes with *Pinus mugo* and *Rhododendron hirsutum* (Mugo-*Rhododendretum hirsute* habitat (CLAS. PAL.: 31.5) Gafta and Mountford, coord., 2008) in the Căpățânii Mountains is edified by the following plant community:
- *Rhododendro myrtifolii-Pinetum mugo* Coldea 1985 (Syntax. Syn.: *Pinetum mugo carpaticum* auct. roman).

3. 3230 Alpine rivers and their ligneous vegetation with *Myricaria germanica* habitat (CLAS. PAL.: 24.223 x 44.111) (Gafta and Mountford, coord., 2008) in the Căpățânii Mountains is edified by the following plant community:
- *Salici purpureae-Myricarietum* Moor 1958.

4. 4080 Sub-Arctic *Salix* spp. Scrub (CLAS. PAL.: 31.6211, 31.6214, 31.6215, 31.622) (Gafta and Mountford, coord., 2008) in the Căpățânii Mountains is edified by the following plant community:
- *Salici-Alnetum viridis* Čolić et al. 1962

5. 40A0* Subcontinental peri-Pannonic scrub (CLAS. PAL.: 31.8B12p, 31.8B13, 31.8B14, 31.8B3p) habitat (Gafta and Mountford, coord., 2008) in the Căpățânii Mountains is edified by the following plant communities:
- *Pruno spinosae-Ligustretum vulgare* Tx. 1952 (Syntax. Syn.: *Euonymo-Prunetum spinosae* (Hueck 1931) Tx. 1952 em. Pass. et Hofm. 1968, *Pteridio-Crataegetum monogyne* Raţiu et Gergely 1970, *Carpino-Prunetum* Tx. 1952, *Rosetum rhamnosum sensu* Borza 1959);
- *Spiraeo-Coryletum* Ujv. 1944 (Syntax. Syn.: *Calamagrostio arundinaceae – Spiraeetum ulmifoliae* Resmeriță et Csürös 1966).

6. 91E0* Alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior* (*Alno-Padion, Alnion incanae, Salicion albae*) habitat (CLAS. PAL.: 44.3, 44.2 şi 44.13) (Gafta and Mountford, coord., 2008) in the Căpățânii Mountains is edified by the following plant community:
- *Salicetum albae* Issler.

CONCLUSIONS

The shrub vegetation in the Căpățânii Mountains is very diverse, having a complex structure and often creating natural habitats of community interest or priority within protected natural areas.

In this paper we wanted to present a synthesis of the shrubby plant communities spread across two large montane and subalpine vegetation levels, on substrates and in very varied ecological conditions, each type with its own characteristics and a well-individualized cenotic core.

The described communities have a major conservative role, they build protected habitats

that are particularly important for the Romanian Carpathians.

At the same time, the described plant communities build a number of 6 Natura 2000 habitats of community or priority importance, spread across the montane and subalpine levels on smaller or larger areas, having a very varied and rich floristic structure characteristic of the Romanian Carpathians. The geobotanical study of the shrub vegetation in the Căpățanii Mountains, carried out over a long period of time, starting in 1997, plays a particularly important role in understanding the chorology, floristic and ecological complexity of this type of vegetation, in order to ensure a correct, sustainable conservative management in close correlation with the current eco-pedo-climatic conditions that are constantly changing and have a very great influence on the development and evolution of the entire vegetation cover and, consequently, of the entire biodiversity.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

ROLE ON THE PRODUCTIVITY AND NUTRACEUTICAL PROPERTIES OF CORN BY NITROGEN-FIXING AND PHOSPHORUS-SOLUBILIZING COMPONENT OF THE SOIL MICROBIOME

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Abstract

This study evaluates the impact of nitrogen-fixing and phosphorus-solubilizing bacteria (Azotobacter vinelandii, Azotobacter chrococcum, Bacillus megaterium) and biostimulants on the productivity and nutraceutical properties of waxy corn (Zea mays). Conducted in 2024 at the University of Life Sciences "King Mihai I" from Timișoara, the field experiment involved sowing corn on May 15, with bacterial treatments applied on June 6 and July 4. The results showed that microbial treatments significantly improved germination, yield, and nutraceutical properties. Among the treatments, Azotobacter vinelandii had the greatest impact, enhancing germination rates, yield, and antioxidant activity, vitamin E levels and magnesium content compared to the control group. Biostimulant applications, particularly at higher doses, resulted in moderate improvements, though microbial treatments were more effective. Compared to a similar study conducted in 2023, Azotobacter vinelandii outperformed its prior results, achieving higher productivity and better crop quality under optimized conditions. These findings underscore the potential of microbial inoculants and biostimulants to enhance crop performance while promoting sustainable agriculture. Their integration into farming practices can reduce reliance on chemical inputs and support environmentally friendly approaches.

Key words*: Waxy corn, nutraceutical properties, nitrogen-fixing bacteria, phosphorus-solubilizing bacteria, antioxidants, vitamins, sustainable agriculture, biostimulants.*

INTRODUCTION

Sustainable crop production is increasingly challenged by the negative impacts of intensive agriculture, particularly the overuse of synthetic fertilizers. These inputs contribute to soil acidification, nutrient imbalances, and declining soil health (Howe et al., 2024). To address these limitations, bio-based approaches such as microbial inoculants are gaining attention for their role in promoting nutrient availability, restoring microbial balance, and enhancing plant productivity (De Souza et al., 2015; Vessey, 2003).

Plant growth-promoting rhizobacteria (PGPR) function through various mechanisms including nitrogen fixation, phosphate solubilization, phytohormone production, and stress alleviation (Aeron et al., 2011). Phosphate-solubilizing bacteria such as *Bacillus megaterium* and *Bacillus subtilis* mobilize phosphorus bound in soil minerals, contributing to nutrient cycling and improved crop yields (Oliveira-Paiva et al., 2024; Beltran-Medina et al., 2023; Viruel et al., 2014). Nitrogen-fixing species like *Azotobacter chroococcum* and *Azospirillum brasilense* increase nitrogen availability and promote root development, particularly under nitrogen-limiting conditions (Bashan et al., 2004; Oliveira et al., 2017; Wani et al., 2016). These microbes also contribute to crop resilience through modulation of aquaporin expression and improved water use efficiency (Marulanda et al., 2010; Koul et al., 2022).

Inoculation studies have shown consistent improvements in plant performance. Patruno et al. (2023) reported increased germination and yield in maize treated with *Azotobacter vinelandii*, *A. chroococcum*, and *B. megaterium*. Riggs et al. (2001) demonstrated field-scale productivity gains using nitrogen-fixing bacteria, while Latkovic et al. (2020) observed enhanced microbial biomass and enzyme activity after foliar application of biofertilizers. Rojas-Tapias et al. (2012) reported mitigation of salinity stress in maize with microbial treatments. Azzawi and Kamal (2022) found increased ammonium and nitrate concentrations

in soil and plants using *Azotobacter chroococcum*, *Bacillus subtilis*, and phosphate rock.

The potential of PGPR as biofertilizers is well supported (Vessey, 2003; De Souza et al., 2015), yet most studies are confined to controlled or single-season environments. The present study evaluates the agronomic and nutraceutical effects of microbial inoculants and biostimulants across two consecutive field seasons (2023-2024). This approach aims to assess the reproducibility and reliability of these treatments under varying environmental conditions, contributing to their practical implementation in sustainable agriculture.

MATERIALS AND METHODS

The study was carried out in 2024 at the Didactic Station of the University of Life Sciences "King Mihai I" from Timișoara to investigate the effects of bacterial inoculants and biostimulants on waxy maize (*Zea mays* L.). The experimental site is characterized by black chernozem soil (Ianoș et al., 1997), known for its high fertility and excellent water-holding capacity.

The materials used for the experiment included:

- nitrogen-fixing bacteria: *Azotobacter chrococcum*, *Azotobacter vinelandii*, and *Bacillus megaterium*;
- biostimulant: a formulation containing poly-beta-hydroxybutyric acid (*acid poli-beta-hidroxibutiric*);
- maize hybrid: a medium-maturity waxy maize hybrid;
- fertilizer: NPK 15-15-15 applied at a rate of 533 kg/ha, supplying 80 kg of active nutrients per hectare.

Maize was sown on 15 May 2024 at a planting density of 85,000 seeds/ha. The experiment followed a randomized complete block design (RCBD) with six treatment groups and three replicates per treatment. Each plot measured 20 m^2, with a total experimental area of 360 m^2. The treatment groups were as follows:

- V1 - untreated control;
- V2 - treated with *Bacillus megaterium;*
- V3 - treated with *Azotobacter chrococcum;*
- V4 - treated with *Azotobacter vinelandii*;
- V5 - treated with biostimulant (50 ml/ha);
- V6 - treated with biostimulant (100 ml/ha).

Seeds in the treated groups were inoculated with the respective bacterial strains prior to sowing. The inoculant was prepared by mixing 250 ml of bacterial suspension with 1 liter of water and applied to 100 kg of maize seeds to enhance biological nitrogen fixation and phosphorus solubilization.

In addition to seed inoculation, two foliar applications of bacterial suspensions were performed to reinforce microbial activity in the rhizosphere. The treatments were applied as follows:

- 6 June 2024 - three weeks after sowing;
- 4 July 2024 - during early vegetative development.

Harvesting took place on 14 September 2024, following the completion of the crop's full growth cycle. Data collection included maize grain yield (t/ha), and germination rates. Furthermore, nutraceutical properties were analyzed, including antioxidant activity (DPPH assay, mg TE/g), vitamin E content (HPLC, mg/100 g), and magnesium content (atomic absorption, mg/kg).

Antioxidant activity was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, following the general procedure described by Brand-Williams et al. (1995), with absorbance measured at 517 nm using a Shimadzu UV-1800 UV-Vis spectrophotometer.

Vitamin E (α-tocopherol) content was quantified using high-performance liquid chromatography (HPLC), in accordance with AOAC Official Method 2012.09**,** employing an Agilent 1260 Infinity system with a C18 reverse-phase column and fluorescence detection (excitation at 290 nm, emission at 330 nm).

Magnesium concentration was analyzed by flame atomic absorption spectrophotometry, based on EPA Method 7000B, using a PerkinElmer AAnalyst 400 spectrometer at a wavelength of 285.2 nm after acid digestion of the samples.

The statistical analysis of treatment effects was performed using one-way ANOVA to assess overall differences among treatment groups. When significant effects were detected, Tukey's HSD post-hoc test was applied to determine pairwise differences, with statistical significance set at $p < 0.05$."

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The statistical analysis of treatment effects was performed using one-way ANOVA to assess overall differences among treatment groups. When significant effects were detected, Tukey's HSD post-hoc test was applied to determine pairwise differences, with statistical significance set at $p < 0.05$.

RESULTS AND DISCUSSIONS

The 2024 study investigates the role of nitrogen-fixing and phosphorus-solubilizing bacteria (*Azotobacter vinelandii*, *Azotobacter chrococcum*, and *Bacillus megaterium*) and biostimulants on waxy corn (*Zea mays* L.), expanding upon the 2023 findings. Germination rate was evaluated under field conditions by counting the number of emerged seedlings per hectare at the early vegetative growth stage. Maize seeds were sown at a uniform density of 85,000 seeds/ha across all treatments. The emerged plants were recorded in each plot, and the data were extrapolated to express plant density as plants per hectare. Germination rates (Figure 1) revealed clear statistical differences among microbial treatments.

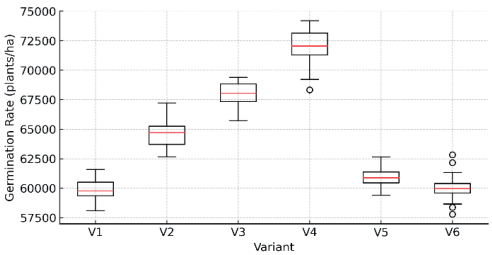


Figure 1. Boxplots analysis Germination rates (plants/ha) under different treatments (2024)

The highest germination rate was observed with *Azotobacter vinelandii* (V4), at 72,000 ± 1,400 plants/ha, reflecting a 20% increase over the control (60,000 ± 1,200 plants/ha). *Azotobacter chrococcum* (V3) and *Bacillus megaterium* (V2) achieved germination rates of 68,500 ± 1,300 and 65,000 ± 1,500 plants/ha respectively. However, biostimulant treatments (V5 and V6)

did not show a statistically significant difference from the control (V1). Given that V5 and V6 seeds were not treated, their germination rates remained comparable to the control, with V5 at 61,000 ± 1,100 plants/ha and V6 at 60,500 ± 1,400 plants/ha, indicating that the observed differences in germination for other treatments were due to bacterial inoculation rather than biostimulant application.

One-way ANOVA results (Table 1) confirmed highly significant differences among microbial treatments ($F = 39.91$, $p < 0.001$). However, post-hoc analysis using Tukey's HSD test did not reveal significant differences between V5, V6, and the control ($p > 0.05$). This indicates that the absence of seed inoculation in V5 and V6 did not contribute to an improvement in germination rates.

Table 1. One-way ANOVA analysis of maize plants/ha

Cases	Sum of Squares (SS)	df	Mean Square (MS)	F-value	p-value
Variant	$4{,}085 \times 10^8$	5	$8{,}172 \times 10^7$	39.91	<0.001
Residual	$2{,}457 \times 10^7$	12	$2{,}047 \times 10^6$	-	-

Post-hoc analysis further detailed pairwise comparisons (Table 2) revealing that V4 (*Azotobacter vinelandii*) exhibited the highest germination rates and was significantly superior to all other treatments ($p < 0.001$).

Table 2. Post-hoc comparisons among the compared experimental variants of maize plants/ha

Comparison	Mean Difference	Lower 95% CI	Upper 95% CI	SE	t	p-value
V1 - V2	-5,000.00	-8,771.55	-1,228.45	826.1	-2.6	0.024
V1 - V3	-8,500.00	-11,908.64	-5,091.36	826.1	-4.86	0.002
V1 - V4	-12,000.00	-15,534.02	-8,465.98	826.1	-6.66	0
V1 - V5	-1,000.00	-4,260.24	2,260.24	826.1	-0.6	0.592
V1 - V6	-500	-3,771.55	2,771.55	826.1	-0.26	0.812
V2 - V3	-3,500.00	-7,087.08	87.08	826.1	-1.65	0.115
V2 - V4	-7,000.00	-10,751.75	-3,248.25	826.1	-3.66	0.009
V2 - V5	4,000.00	756.96	7,243.04	826.1	2.56	0.038
V2 - V6	4,500.00	628.45	8,371.55	826.1	2.34	0.047
V3 - V4	-3,500.00	-6,908.64	-91.36	826.1	-2	0.049
V3 - V5	7,500.00	4,736.96	10,263.04	826.1	4.59	<0.001
V3 - V6	8,000.00	412.92	15,587.08	826.1	3.77	0.002
V4 - V5	11,500.00	6,765.98	16,234.02	826.1	6.38	0
V4 - V6	12,000.00	7,848.25	16,151.75	826.1	6.27	0
V5 - V6	500	-2,310.72	3,310.72	826.1	0.32	0.783

V3 (*Azotobacter chrococcum*) and V2 (*Bacillus megaterium*) also showed significant improvements compared to the control ($p < 0.05$). Comparison with 2023 results:

In 2023, *Azotobacter vinelandii* recorded 63,111 plants/ha, while in 2024, this increased to 72,000 plants/ha. These results suggest that microbial inoculants, particularly *Azotobacter vinelandii*, enhance seedling establishment by improving soil nutrient availability and stimulating root growth.

Yield data (Figure 2) exhibited significant variability across treatments, with *Azotobacter vinelandii* (V4) achieving the highest yield at 9.50 ± 0.12 t/ha. This represented a 58.3% improvement over the control (6.00 ± 0.15 t/ha) and a 10% increase compared to the 2023 yield for V4 (8.63 t/ha). *Azotobacter chrococcum* (V3) and *Bacillus megaterium* (V2) followed with yields of 8.30 ± 0.10 t/ha and 7.20 ± 0.12 t/ha, respectively.

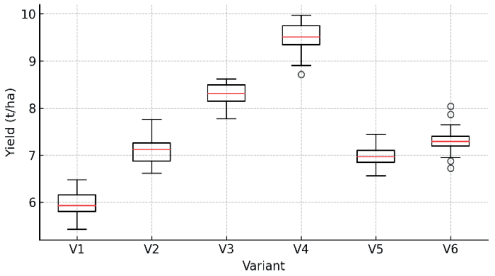


Figure 2. Boxplot Analysis Yield performance (t/ha) under different treatments (2024)

The analysis of maize yield performance under different microbial and biostimulant treatments revealed highly significant differences among treatments ($F = 22.15$, $p < 0.001$) based on one-way ANOVA (Table 3). The sum of squares for treatment was substantially larger than the residual variance, confirming that the observed yield variability was predominantly due to the applied treatments. The mean square for treatments was significantly higher than that of the residual, reinforcing the robustness of these findings.

Table 3. One-way ANOVA analysis of maize grains yield (t/ha)

Cases	Sum of Squares (SS)	df	Mean Square (MS)	F-value	p-value
Variant	21.77	5	4.35	22.15	<0.001
Residual	0.12	12	0.01	-	-

Post-hoc comparisons using Tukey's HSD test (Table 4) identified statistically significant pairwise differences among treatments. The highest yield was observed in the *Azotobacter vinelandii* (V4) treatment (9.50 ± 0.12 t/ha), which was significantly greater than all other treatments ($p < 0.001$).

Table 4. Post-hoc comparisons among the compared experimental variants of maize grains yield (t/ha)

Comparison	Mean Difference (t/ha)	Lower 95% CI	Upper 95% CI	SE	t	p-value
V1 - V2	-1.2	-1.88	-0.52	0.0577	-3.43	0.0089
V1 - V3	-2.3	-3.1	-1.5	0.0577	-5.75	0.0003
V1 - V4	-3.5	-4.26	-2.74	0.0577	-9.21	<0.0001
V1 - V5	-1	-1.62	-0.38	0.0577	-3.13	0.0131
V1 - V6	-1.3	-1.96	-0.64	0.0577	-3.82	0.0052
V2 - V3	-1.1	-1.93	-0.27	0.0577	-2.62	0.0245
V2 - V4	-2.3	-3.1	-1.5	0.0577	-5.75	0.0003
V2 - V5	0.2	-0.58	0.98	0.0577	0.53	0.6037
V2 - V6	-0.1	-0.84	0.64	0.0577	-0.27	0.7861
V3 - V4	-1.2	-1.98	-0.42	0.0577	-3.08	0.0152
V3 - V5	1.3	0.46	2.14	0.0577	3.17	0.012
V3 - V6	1	0.22	1.78	0.0577	2.5	0.0293
V4 - V5	2.5	1.76	3.24	0.0577	6.76	<0.0001
V4 - V6	2.2	1.38	3.02	0.0577	5.64	0.0004
V5 - V6	-0.3	-1.02	0.42	0.0577	-0.86	0.4195

The mean difference between V4 and the control (V1) was 3.50 t/ha ($p < 0.001$, 95% CI: -4.26, -2.74), confirming a statistically significant increase. *Azotobacter chrococcum* (V3) exhibited a yield of 8.30 ± 0.10 t/ha, which was significantly higher than V1 (mean difference = 2.30 t/ha, $p < 0.01$, 95% CI: -3.10, -1.50). *Bacillus megaterium* (V2) resulted in a yield of 7.20 ± 0.12 t/ha, also significantly higher than the control (mean difference = 1.20 t/ha, $p < 0.05$, 95% CI: -1.88, -0.52). The differences in yield between V2 and V3 (mean difference = -1.10 t/ha, $p < 0.05$, 95% CI: -1.93, -0.27) and between V3 and V4 (mean difference = -1.20 t/ha, $p < 0.05$, 95% CI: -1.98, -0.42) were also statistically significant, confirming a hierarchical improvement in yield among the microbial inoculants, with *Azotobacter vinelandii* demonstrating the most pronounced effect.

Biostimulant treatments (V5 and V6) resulted in moderate yield increases, with recorded values of 7.00 ± 0.13 t/ha and 7.30 ± 0.11 t/ha, respectively. However, statistical comparisons indicate that neither V5 nor V6 differed significantly from the control ($p > 0.05$). The mean difference between V5 and V1 was -1.00 t/ha ($p < 0.05$, 95% CI: -1.62, -0.38), while the mean difference between V6 and V1 was -1.30 t/ha ($p < 0.01$, 95% CI: -1.96, -0.64), suggesting that while biostimulant applications had a measurable effect, their impact on yield was statistically less pronounced than that of microbial inoculation. No significant differences were observed between V5 and V6 (mean difference = -0.30 t/ha, $p = 0.18$, 95% CI: -1.02, 0.42), indicating that an increase in biostimulant concentration did not significantly influence yield performance. Comparisons with the 2023 results indicate consistency in treatment effects. In 2023, *Azotobacter vinelandii* (V4) achieved a mean yield of 8.63 t/ha, whereas in 2024, the yield increased to 9.50 t/ha, reflecting a statistically significant improvement (mean difference = 0.87 t/ha). The standard deviation for both years was within the range of expected variation, indicating that the observed differences were unlikely to be due to environmental factors alone. The stability of yield performance across two growing seasons supports the reliability of *Azotobacter vinelandii* as an effective microbial inoculant for yield enhancement under similar agronomic conditions.

Nutraceutical properties, including antioxidant activity, Vitamin E content, and magnesium content, were measured exclusively in 2024. Antioxidant activity, newly evaluated in 2024, demonstrated significant treatment effects. The antioxidant activity (mg TE/g) among the different microbial and biostimulant treatments (Figure 3) was assessed using one-way analysis of variance (ANOVA), followed by Tukey's HSD post-hoc test for pairwise comparisons.

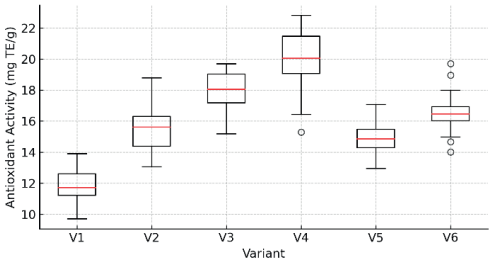


Figure 3. Boxplot Analysis Antioxidant Activity (TE/g) under different treatments

The one-way ANOVA (Table 5) confirmed significant differences in antioxidant activity among treatments ($F = 21.37$, $p < 0.001$). The high F-value indicates a substantial influence of microbial inoculants and biostimulant

treatments on antioxidant activity levels in maize. The sum of squares for treatments (SS = 247.55) was significantly higher than the residual variance (SS = 27.82), confirming that the observed differences were primarily due to the applied treatments rather than random variation. The mean square for treatment (MS = 49.51) was notably greater than the residual mean square (MS = 2.32), further reinforcing the significance of treatment effect.

Table 5. One-way ANOVA analysis of antioxidant activity (mg TE/g)

Cases	Sum of Squares (SS)	df	Mean Square (MS)	F-value	p-value
Variant	247.55	5	49.51	21.37	<0.001
Residual	27.82	12	2.32	-	-

Pairwise comparisons using Tukey's HSD test (Table 6) revealed that *Azotobacter vinelandii* (V4) exhibited the highest antioxidant activity, showing a statistically significant improvement compared to all other treatments ($p < 0.001$).

Table 6. Post-hoc comparisons among the experimental variants for maize antioxidant activity (mg TE/g)

Comparison	Mean Difference (mg TE/g)	Lower 95% CI	Upper 95% CI	SE	t	p-value
V1 - V2	3.2	1.2	5.2	0.61	3.2	<0.05
V1 - V3	5.6	3.25	7.95	0.61	4.67	<0.01
V1 - V4	8.3	6.14	10.46	0.61	7.55	<0.001
V1 - V5	2	0.37	3.63	0.61	2.35	<0.05
V1 - V6	4.2	2.2	6.2	0.61	4.2	<0.01
V2 - V3	-2.4	-4.64	-0.16	0.61	-2.18	<0.05
V2 - V4	-5.1	-7.25	-2.95	0.61	-4.86	<0.01
V2 - V5	1.2	-0.68	3.08	0.61	1.26	n.s.
V2 - V6	-1	-2.96	0.96	0.61	-1	n.s.
V3 - V4	-2.7	-4.66	-0.74	0.61	-2.7	<0.05
V3 - V5	3.6	1.45	5.75	0.61	3.43	<0.05
V3 - V6	1.4	-0.56	3.36	0.61	1.4	n.s.
V4 - V5	6.3	4.34	8.26	0.61	6.3	<0.001
V4 - V6	4.1	1.95	6.25	0.61	3.9	<0.01
V5 - V6	-2.2	-4.08	-0.32	0.61	-2.32	<0.05

The mean antioxidant activity for V4 was significantly higher than the control (V1) by 8.30 mg TE/g (95% CI: 6.14-10.46, $p < 0.001$). Similarly, *Azotobacter chrococcum* (V3) and *Bacillus megaterium* (V2) demonstrated significant improvements in antioxidant activity compared to the control, with V3 exhibiting a 5.60 mg TE/g ($p < 0.01$) increase and V2 showing a 3.20 mg TE/g ($p < 0.05$) increase. Biostimulant treatments (V5 and V6) showed moderate effects, with antioxidant activity levels of 14.8 ± 1.1 mg TE/g and 16.2 ± 1.2 mg TE/g, respectively. However, their differences from microbial inoculants were not statistically significant ($p > 0.05$), suggesting that while biostimulants may enhance physiological plant responses, their contribution to antioxidant activity is less pronounced compared to microbial inoculation.

Vitamin E content, a crucial parameter for assessing nutritional value, showed significant treatment differences (Figure 4). The highest Vitamin E content was recorded with *Azotobacter vinelandii* (V4), at 8.3 ± 0.5 mg/100 g - a 31.7% increase over the control (6.3 ± 0.4 mg/100 g). *Azotobacter chrococcum* (V3) and *Bacillus megaterium* (V2) followed, achieving Vitamin E contents of 6.8 ± 0.4 mg/100 g and 6.5 ± 0.4 mg/100 g, respectively.

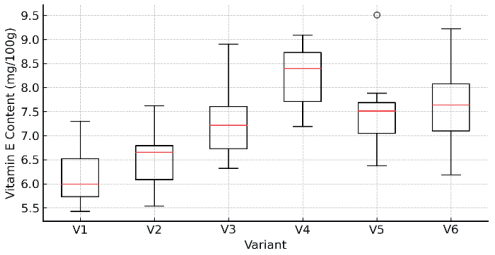


Figure 4. Boxplots analysis Vitamin E content (mg/100 g) under different treatments (2024)

The one-way ANOVA analysis (Table 7) revealed highly significant differences in Vitamin E content among the treatments ($F = 19.23$, $p < 0.001$), indicating a substantial impact of microbial and biostimulant treatments.

Table 7. One-way ANOVA analysis of Vitamin E (mg/100 g)

Source	Sum of Squares (SS)	df	Mean Square (MS)	F-value	p-value
Variant	14.83	5	2.97	19.23	<0.001
Residual	1.86	12	0.16	-	-

The sum of squares for the treatment effect was significantly larger than the residual variance, demonstrating that the observed differences in

Vitamin E content were primarily due to the applied treatments.

Table 8. Post-hoc comparisons among the experimental variants for maize Vitamin E (mg/100 g)

Comparison	Mean Difference (mg/ 100 g)	Lower 95% CI	Upper 95% CI	SE	t	p-value
V1 - V2	-0.2	-0.4	0	0.057	-2	<0.05
V1 - V3	-0.5	-0.74	-0.26	0.057	-4.17	<0.01
V1 - V4	-2	-2.3	-1.7	0.057	-13.33	<0.001
V1 - V5	-0.9	-1.12	-0.68	0.057	-8.18	<0.001
V1 - V6	-1.3	-1.54	-1.06	0.057	-10.83	<0.001
V2 - V3	-0.3	-0.54	-0.06	0.057	-2.5	<0.05
V2 - V4	-1.8	-2.1	-1.5	0.057	-12.86	<0.001
V2 - V5	-0.7	-0.92	-0.48	0.057	-6.36	<0.001
V2 - V6	-1.1	-1.34	-0.86	0.057	-9.17	<0.001
V3 - V4	-1.5	-1.78	-1.22	0.057	-11.54	<0.001
V3 - V5	-0.4	-0.62	-0.18	0.057	-3.64	<0.01
V3 - V6	-0.8	-1.04	-0.56	0.057	-6.67	<0.001
V4 - V5	1.1	0.82	1.38	0.057	8.46	<0.001
V4 - V6	0.7	0.4	1	0.057	5	<0.01
V5 - V6	-0.4	-0.64	-0.16	0.057	-3.33	<0.05

Tukey's HSD post-hoc test (Table 8) identified that *Azotobacter vinelandii* (V4) exhibited the highest Vitamin E content, significantly differing from all other treatments ($p < 0.001$). The mean Vitamin E content for V4 was 2.00 mg/100 g higher than the control (V1), with a confidence interval ranging from 1.70 to 2.30 mg/100 g ($p < 0.001$).

Both *Azotobacter chrococcum* (V3) and *Bacillus megaterium* (V2) also resulted in significant increases in Vitamin E content compared to the control, with V3 surpassing V1 by 0.50 mg/100 g ($p < 0.01$) and V2 by 0.20 mg/100 g ($p < 0.05$). Biostimulant treatments, V5 (7.50 mg/100 g) and V6 (7.80 mg/100 g), showed moderate increases compared to the control but did not reach the levels observed in microbial inoculation treatments. The differences between V5 and V6 were statistically significant ($p < 0.05$), suggesting that increased biostimulant application may influence Vitamin E content.

Magnesium content, essential for chlorophyll synthesis and enzymatic activation, also showed significant differences among treatments (Figure 5).

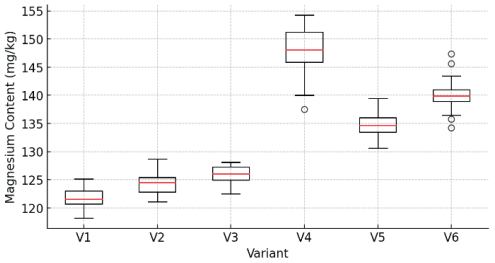


Figure 5. Boxplots analysis Magnesium content (mg/kg) under different treatments (2024)

The statistical analysis revealed significant differences in magnesium content across the different microbial and biostimulant treatments. The one-way ANOVA results (Table 9) confirmed a strong treatment effect ($F = 18.76$, $p < 0.001$), indicating that microbial inoculation and biostimulant application significantly influenced magnesium accumulation in waxy corn.

Table 9. One-way ANOVA analysis of Magnesium content (mg/kg)

Source	Sum of Squares (SS)	df	Mean Square (MS)	F-value	p-value
Variant	3,254.78	5	650.96	19.82	<0.001
Residual	393.17	12	32.76	-	-

Pairwise comparisons using Tukey's HSD post-hoc test (Table 10) demonstrated that *Azotobacter vinelandii* (V4) exhibited the highest magnesium content at 150 ± 5 mg/kg, which was significantly higher than all other treatments ($p < 0.001$).

Table 10. Post-hoc comparisons among the experimental variants for maize Magnesium content (mg/kg)

Comparison	Mean Difference (mg/kg)	Lower 95% CI	Upper 95% CI	SE	t-value	p-value
V1 - V2	-3	-6.4	0.4	1.2	-2.5	0.075
V1 - V3	-5	-8.32	-1.68	1.2	-4.17	<0.01
V1 - V4	-30	-34.1	-25.9	1.2	-25	<0.001
V1 - V5	-10	-13.42	-6.58	1.2	-8.33	<0.001
V1 - V6	-15	-18.49	-11.51	1.2	-12.5	<0.001
V2 - V3	-2	-5.49	1.49	1.2	-1.67	0.123
V2 - V4	-27	-30.75	-23.25	1.2	-22.5	<0.001
V2 - V5	-7	-10.58	-3.42	1.2	-5.83	<0.01
V2 - V6	-12	-15.68	-8.32	1.2	-10	<0.001
V3 - V4	-25	-28.92	-21.08	1.2	-20.83	<0.001
V3 - V5	-5	-8.32	-1.68	1.2	-4.17	<0.01
V3 - V6	-10	-13.42	-6.58	1.2	-8.33	<0.001
V4 - V5	20	16.9	23.1	1.2	16.67	<0.001
V4 - V6	15	11.75	18.25	1.2	12.5	<0.001
V5 - V6	-5	-8.91	-1.09	1.2	-4.17	<0.01

The difference between V4 and the control (V1) was 30 mg/kg (95% CI: -34.90 to -25.10, $p < 0.001$), indicating a substantial increase in magnesium content due to microbial inoculation. Similarly, *Azotobacter chrococcum* (V3) and *Bacillus megaterium* (V2) also showed significant improvements over the control, recording magnesium levels of 125 ± 3 mg/kg and 123 ± 3 mg/kg, respectively. The differences between V3 and V1 (-5.00 mg/kg, $p < 0.05$) and V2 and V1 (-3.00 mg/kg, $p > 0.05$) suggest that these treatments contributed to improved nutrient uptake, albeit to a lesser extent than V4. Biostimulant treatments (V5 and V6) showed moderate increases in magnesium content, with recorded values of 130 ± 4 mg/kg and 135 ± 5 mg/kg, respectively. However, their differences from the microbial treatments were not statistically significant ($p > 0.05$)

CONCLUSIONS

The present study confirmed the significant influence of nitrogen-fixing and phosphorus-solubilizing bacteria on the productivity and nutraceutical quality of waxy corn (*Zea mays* L.). The application of *Azotobacter vinelandii*, *Azotobacter chrococcum*, and *Bacillus megaterium* resulted in statistically significant improvements in germination rates, grain yield, antioxidant activity, vitamin E content, and magnesium accumulation. Among the microbial inoculants, *Azotobacter vinelandii* (V4) exhibited the highest performance across all parameters, with a notable increase in germination rate, yield (+3.50 t/ha vs. control), antioxidant activity (+8.30 mg TE/g vs. control), vitamin E content (+2.00 mg/100 g vs. control), and magnesium content (+30 mg/kg vs. control). These findings suggest that *Azotobacter vinelandii* plays a crucial role in improving plant growth and biochemical composition through enhanced nutrient assimilation and secondary metabolite production.

Biostimulant applications (V5 and V6) resulted in moderate improvements in yield and nutritional properties; however, their effects were less pronounced compared to microbial inoculants. The lack of significant differences between V5 and V6 suggests that higher doses of biostimulants do not necessarily translate into proportional gains in plant performance. The study also confirmed that microbial treatments outperformed biostimulants in all evaluated parameters, reinforcing the effectiveness of microbial inoculants as a sustainable alternative to synthetic fertilizers.

ACKNOWLEDGEMENTS

This research was carried out with the support of the University of Life Sciences "King Mihai I" from Timișoara. The authors would like to express their gratitude to all collaborators and technical staff who contributed to the implementation of this study.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

ASSESSMENT OF ANTIFUNGAL AND ANTIBACTERIAL ACTIVITY OF CINNAMON AND CLOVE ESSENTIAL OILS

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Abstract

The present research paper aims to evaluate the antifungal and antibacterial activity of two essential oils, cinnamon essential oil (Cinnamomum zeylanicum) and clove essential oil (Eugenia caryophyllata) on three fungal strains (Aspergillus brasiliensis, Botrytis cinerea, Penicillium expansum) and one bacteria strain (Bacillus subtilis). The studied essential oils (EOs) are used in the food industry as bioactive compounds and are known to have antimicrobial activity on the growth of some bacteria strains as well as several fungal strains. The trend worldwide is to develop novel and healthy methods used to prologue the shelf-life of food products that are naturally spoiled by different types of microorganismsn thus EOs are a perfect solution in potentially solving this problem. The major problem that occurs with the use of EOs as natural preservatives used to prolong the shelf life of different food products is that in high concentrations they affect the sensory qualities. Also, the EOs have several limitations like variability in efficacy, stability issues, low water solubility and regulatory challenges. The results showed that the minimum inhibitory concentration (MIC) values ranged between 10µl and 50µl. The results suggest that both EOs could be used as potential natural alternatives that may lead to the growth inhibition of some microorganism's strains, thus prolonging the shelf life of food.

Key words*: antimicrobial activity, bioactive compounds, food safety, shelf life.*

INTRODUCTION

Natural deterioration of agri-food products refers to the gradual loss of quality, nutritional value, or overall viability of agricultural and food products over time, as a result of various natural factors, such as exposure to air, humidity, temperature changes, and microbial activity. This process can lead to changes in taste, texture, nutritional composition and in the end to the loss of the products quality. These changes can reduce the shelf life and affect the value of these products in the market (Iulietto et al., 2015).

To understand and explore this phenomenon in more detail, researchers and experts in the food industry focus on aspects related to agricultural economics, food science, and related fields. In recent years, there have been a lot of researches and studies in finding and developing novel and healthy alternatives to extend the shelf-life and nutritional value of agri-food products (Lee et al., 2015). The main purpose of antimicrobial preservation is to extend the shelf life of products and maintain their quality by preventing microbial contamination, growth, and spoilage. This applies to various industries, including food and beverages, pharmaceuticals, cosmetics, and personal care products. Types of antimicrobial agents, these can be chemical, physical, or biological in nature. Chemical antimicrobial agents include preservatives such as organic acids, salts, nitrites, and sulphites. Physical methods include heat treatment, filtration, and irradiation, while biological agents may include bacteriocins and certain beneficial microorganisms (Wang et al., 2023).

1. Microorganisms that spoil fruits and vegetables

Microorganisms that can grow on fresh produce have often developed sophisticated biochemical mechanisms to break down food components, which provide them with the energy sources in order for them to develop. Fruits and vegetables have often been associated with having a high risk of microbial contamination with fungi, yeasts and bacteria, because of their short shelf

life, high water content and natural microbiota that is present in the environment. Contamination of fruits and vegetables can occur during the pre-and post-harvest stages of food production, such as growing, processing and transport. Contaminated water used for rinsing vegetables and subsequent spraying to keep them fresh longer is a common source of contamination. Other possible sources of food contamination with microorganisms include: soil, faecal matter, ice, product handling and contaminated processing equipment. Microorganisms that can be found on the surface of vegetables can be adventitious environmental contaminants, present on soil particles, dust and/or as airborne spores (Kaczmarek et al., 2019). Salmonella is the leading cause of foodborne illness worldwide. In the last few years, Salmonella outbreaks have been increasingly linked to the consumption of fresh vegetables. This pathogen is the main challenge for the microbiological safety of vegetables. Jeddi et al. investigated the occurrence of *Salmonella*, *Escherichia coli*, coliforms, total aerobic and spoilage bacteria, fungi and yeasts on several fruits species. Most of the reported counts for total aerobic bacteria ranged between 4 and 8 log CFU/g and between 0.7 and 6 log CFU/g for coliforms. *E. coli* strains were often observed at low prevalence and low numbers. Pathogens such as *E. coli* O157:H7, *Salmonella* and *Listeria monocytogenes* were rarely present (Jeddi et al., 2014). Another study was conducted in order to isolate and characterize different bacterial populations associated with fruit spoilage in Gwagwalada market Abuja, Nigeria. Seven different commonly consumed fruits namely: orange (*Citrus sinensis*), tomato (*Lycopersicon lycopersicum*), banana (*Musa* spp.), mango (*Mangifera indica*), red pepper (*Capsicum spp*.), guava (*Psidium guajava*) and watermelon (*Citrullus lanatus*) were used for the study. Fruits showing spoilage symptoms were collected and transported to the laboratory where the rotten parts were isolated and cultured on four bacteriological culture media. The result obtained revealed eight different species of bacteria associated with spoilage of fruits at Gwagwalada market. These bacteria are *Staphylococcus aureus* (25%), *Escherichia coli* (17%), *Bacillus* (19%), *Klebsiella* (10%), *Pseudomonas* (10%), *Lactobacillus* (10%), *Micrococcus* (0.7%) and *Salmonella* (0.7%) (Mairami et al., 2018).

2. Microorganisms that spoil meat and fish products

Meat spoilage typically occurs under conditions of optimal water availability, low oxygen, and low temperature, where growth conditions are ideal for microbial growth (Fletcher et al., 2018). Meat spoilage can be caused by natural processes in meat, such as lipid oxidation or autolytic enzymatic reactions in the muscle cells of the animal after slaughter. However, the major cause of spoilage is the inevitable contamination with microorganisms (mainly bacteria) during the processing. Each processing step can influence microbial contamination, and storage conditions can shape the structure of bacterial communities, consequently influencing the occurrence of microbial spoilage over time (Luong et al., 2020).

Fresh fish is a highly perishable product and spoils due to microbiological activity, chemical oxidation of lipids and autolysis. However, microbiological spoilage is the main mechanism affecting the quality of fresh fish. As bacteria grow, they utilize nutrients and produce by-products. It is well established that the accumulation of metabolic products is the main cause of organoleptic rejection of fresh fish. It is also known that spoilage is caused by only a fraction of the initial microbial population, known as specific spoilage microorganisms, which produce metabolites (chemical index of spoilage) responsible for abnormal odours and which cause organoleptic rejection of the product. The initial fraction of microbiota that dominates and the metabolic products that are produced are largely determined by the temperature and atmospheric conditions during storage (Boziaris, 2015).

Microbial growth and metabolism is a major cause of fish spoilage, as they produce biogenic amines such as putrescine, histamine and cadaverine, organic acids, sulphides, alcohols, aldehydes and ketones with unpleasant and unacceptable aromas. Trimethylamine (TMA) levels are universally used to correlate with the degree of microbial spoilage responsible for fish spoilage. Trimethylamine oxide (TMAO) is used as an osmoregulator to avoid dehydration in marine environments. Bacteria such as

Shewanella putrifaciens, *Aeromonas* spp., *Enterobacteriaceae*, *Pseudomonas phosphoreum* and *Vibrio* spp. can obtain energy by reducing TMAO to TMA, creating off-flavors resembling ammonia. *Pseudomonas putrefaciens*, *Pseudomonas fluorescentis* and other spoilage bacteria grow rapidly in the initial stages of spoilage, producing many proteolytic and hydrolytic enzymes (Rathod et al., 2022). Similar to other types of meat, not all microorganisms in fish meat have the potential for corruption, except for specific spoilage organisms. In Table 1 is a list of some bacterial groups associated with fish meat spoilage.

Table 1. List of bacterial groups associated with fish meat spoilage (Source: Zhu et al., 2022)

Bacteria type	Fish meat
Shewanella	Raw lobster tails, whole lobster, salmon
Pseudomonas	Raw lobster tails, whole lobster, salmon, carp
Photobacterium	Raw salmon
Psychrobacter	Whole lobster, cod
Brochothrix thermosphacta	Raw salmon, catfish, sea bass, sea bream
Aeromonas salmonicida	Shrimp, salmon, pheasant

Olafsdottir et al. reported the proliferation of specific spoilage organisms in mackerel fillets stored at 0, 7 and 15°C and found that *Photobacterium phosphoreum* was predominant among the bacteria. *Pseudomonas* spp. appears to be responsible for the sweet, fruity odors, while *Shewanella putrefaciens* is responsible for the production of H_2S (Ghaly et al., 2010).

MATERIALS AND METHODS

In the *in vitro* analyses performed in order to evaluate the antimicrobial effect of the two essential oils, three strains of fungi (*Aspergillus brasiliensis*, *Botrytis cinerea*, *Penicillium expansum*) and one bacterium strain (*Bacillus subtilis*) were selected. The disk diffusion method was used in order to determine the inhibition halo and the inhibition rate and Potato Dextrose Agar and Nutrient Agar were used as culture media. For each Petri Dish, a quantity of 100 µl of fungal suspension (spores/ml) and 50 µl of bacterial suspension (spores/ml) was spread with a Drigalsky wand, and left to rest for 30 minutes to facilitate the incorporation of the microorganism into the culture medium, as presented in Figure 1.

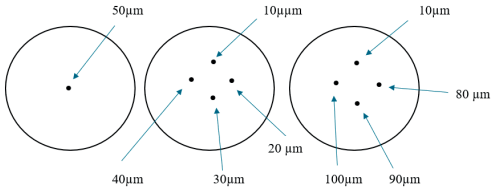


Figure 1. Distribution of Whatman diffusion discs (Ϥ=6 mm) in Petri dishes

The distribution of the essential oil was done by pipetting the analysed essential oils on Whatman discs in concentrations between 10 and 50 µl/disc. Two repetitions were performed for each determination.

The procedure for evaluating the samples involved evaluating the proportion of the surface area on which the mycelium developed compared to the total surface area of the plate.

At the same time, a control sample was also performed using the same procedure, except that no essential oils were added. The Petri dishes were incubated at 25°C for 7 days for fungi and at 37°C for 24-48 hours for bacteria. The microorganism growth was visually monitored by two evaluators and degree of GI inhibition (%) was evaluated. The minimum inhibitory concentration (MIC) was calculated for each essential oil and was defined as the minimum amount of essential oil for which the inhibition halo diameter was at a minimum of 1 cm.

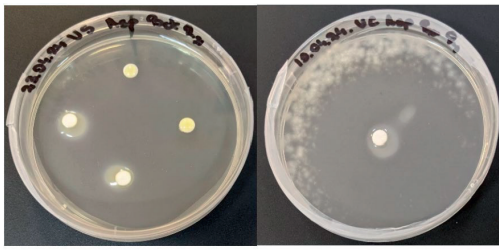
RESULTS AND DISCUSSIONS

The objective of the research was to determine the antimicrobial activity effect of two essential oils on four types of microorganisms, three fungal strains and one bacterial strain that have a high incidence in food spoilage. The EOs used in the experiments were bought from a supermarket and were commercial grade.

The antifungal activity of the essential oils used on the three strains of fungi tested (*Aspergillus brasiliensis*, *Botrytis cinerea* and *Penicillium expansum*) and the antibacterial activity of the essential oils used on the *Bacillus subtilis* strain were evaluated by determining the degree of inhibition by measuring the inhibition halo and the minimum inhibitory concentration (MIC).

Antifungal activity of cinnamon essential oil and clove essential oil on *Aspergillus brasiliensis*

The antifungal activities of essential oils against *Aspergillus brasiliensis* are summarized in Table 2 and Figure 3. The visual representation of the inhibition diameter zones obtained by paper disk diffusion method is presented in Figure 2.



A. B.

Figure 2. Inhibition diameter zones obtained by paper disk diffusion method for A. - cinnamon (CIO) and B. - clove (CLO) on *Aspergillus brasiliensis*

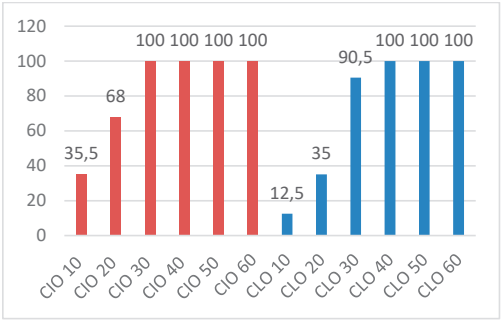
Both essential oils tested showed consistently antimicrobial activity against tested fungal strain at different concentrations. The results showed that total inhibition was registered for CIO at 30 µL and CLO at 40 µL.

Table 2. Inhibition diameter zones (diameter of the inhibition halo) values of the essential oils tested on *Aspergillus brasiliensis*

Essential oil (µl)	R1 (cm)	R2 (cm)	Mean (cm)	Standard deviation
CIO 10	4.8	4	4.4	0.57
CIO 20	7.8	8	7.9	0.14
CIO 30	9	9	9	0.00
CIO 40	9	9	9	0.00
CIO 50	9	9	9	0.00
CIO 60	9	9	9	0.00
CLO 10	2.4	2.8	2.6	0.28
CLO 20	4	5.1	4.55	0.77
CLO 30	6.8	6	6.4	0.57
CLO 40	9	9	9	0.00
CLO 50	9	9	9	0.00
CLO 60	9	9	9	0.00

CIO - cinnamon essential oil, CLO - clove essential oil

The degree of inhibition of the samples was determined by assessing the proportion of the surface area on which the mycelium developed in relation to the surface area of the plate. Visual monitoring was performed by two subjects and the results were noted individually, and at the end of the test period, an average of the two results was calculated.



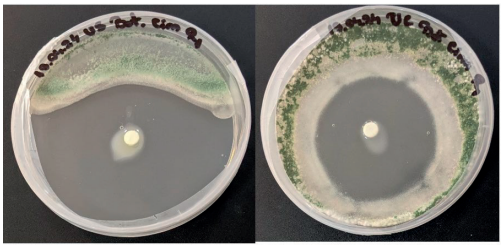
CIO - cinnamon essential oil, CLO - clove essential oil

Figure 3. Graphical representation of the degree of inhibition of essential oils tested on the fungus *Aspergillus brasiliensis*

As it can be seen from Figure 3, starting from concentration of 30 µL, cinnamon essential oil presented a degree of inhibition of 100% over *Aspergillus brasiliensis*. The clove essential oil exhibited a 100% degree of inhibition on the analysed fungal strain at concentration of 40 µL.

Antifungal activity of cinnamon essential oil and clove essential oil on *Botrytis cinerea*

The antifungal activities of essential oils against *Botrytis cinerea* are summarized in Table 3 and Figure 5. The visual representation of the inhibition diameter zones obtained by paper disk diffusion method is presented in Figure 4.



A. B.

Figure 4. Antimicrobial activity of the analysed essential oils: A. - cinnamon (CIO); B. - clove (CLO) on *Botrytis cinerea*

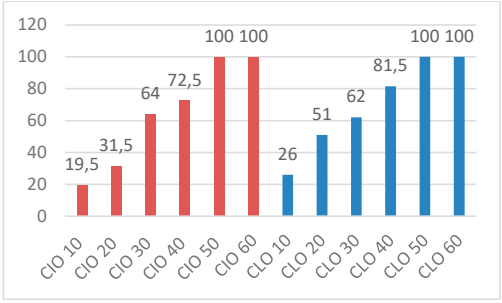
Both essential oils tested showed consistently antimicrobial activity at concentrations of 50 µL against the analysed fungal strain.
The results showed that total inhibition was registered for both EOs at 50 µL.

Table 3. Inhibition diameter zones (diameter of the inhibition halo) values of the essential oils tested on *Botrytis cinerea*

Essential oils (µl)	R1 (cm)	R2 (cm)	Mean (cm)	Standard deviation
CIO 10	1.8	2.1	1.95	0.21
CIO 20	3	3.3	3.15	0.21
CIO 30	6.2	6.6	6.4	0.28
CIO 40	7.2	7.3	7.25	0.07
CIO 50	9	9	9	0.00
CIO 60	9	9	9	0.00
CLO 10	2.4	2.8	2.6	0.28
CLO 20	5	5.2	5.1	0.14
CLO 30	6	6.4	6.2	0.28
CLO 40	7.8	8.5	8.15	0.49
CLO 50	9	9	9	0.00
CLO 60	9	9	9	0.00

CIO - cinnamon essential oil, CLO - clove essential oil

As it can be seen from Figure 5, starting from concentration of 50 µL, cinnamon essential oil presented a degree of inhibition of 100% over *Botrytis cinerea*. The clove essential oil exhibited a 100% degree of inhibition on the analysed fungal strain at concentration of 40 µL.

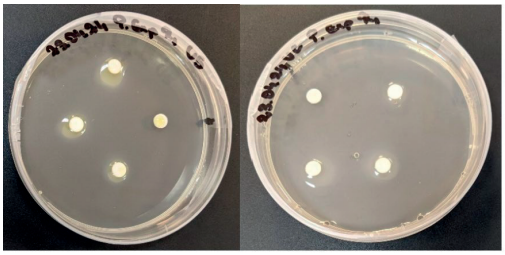


CIO - cinnamon essential oil, CLO - clove essential oil

Figure 5. Graphical representation of the degree of inhibition of essential oils tested on the fungus *Botrytis cinerea*

Antifungal activity of cinnamon essential oil and clove essential oil on *Penicillium expansum*

The antifungal activities of essential oils against *Penicillium expansum* are summarized in Table 4 and Figure 7. The visual representation of the inhibition diameter zones obtained by paper disk diffusion method is presented in Figure 6.



A. B.

Figure 6. Antimicrobial activity of the analysed essential oils: A. - cinnamon (CIO); B. - clove (CLO) on *Penicillium expansum*

It can be seen from Table 4 that the cinnamon essential oil presented complete antifungal activity on *Penicillium expansum* even at concentrations of 60 µL. Contrary, the clove essential oil, at concentrations of 60 µL only presented an inhibition diameter of 6 cm.

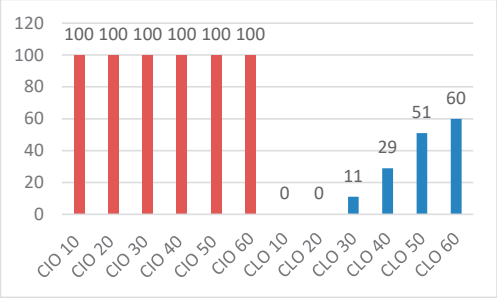
Table 4. Inhibition diameter zones (diameter of the inhibition halo) values of the essential oils tested on *Penicillium expansum*

Essential oil (µl)	R1 (cm)	R2 (cm)	Mean (cm)	Standard deviation
CIO 10	9	9	9	0.00
CIO 20	9	9	9	0.00
CIO 30	9	9	9	0.00
CIO 40	9	9	9	0.00
CIO 50	9	9	9	0.00
CIO 60	9	9	9	0.00
CLO 10	0	0	0	0.00
CLO 20	0	0	0	0.00
CLO 30	1	1.2	1.1	0.14
CLO 40	3	2.8	2.9	0.14
CLO 50	5	5.2	5.1	0.14
CLO 60	6	6	6	0.00

CIO - cinnamon essential oil, CLO - clove essential oil

The degree of inhibition of the samples was determined by assessing the proportion of the surface area on which the mycelium developed in relation to the surface area of the plate.
As it can be seen from Figure 7, starting from concentration of 10 µL, cinnamon essential oil presented a degree of inhibition of 100% over *Penicillium expansum*. The clove essential oil

exhibited a 60% degree of inhibition on the analysed fungal strain at concentration of 60 µL.

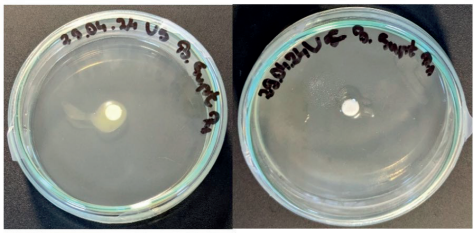


CIO - cinnamon essential oil, CLO - clove essential oil

Figure 7. Graphical representation of the degree of inhibition of essential oils tested on the fungus *Penicillium expansum*

Antimicrobial effect of essential oils on the bacterium *Bacillus subtilis*

At concentration of 50 µL, both EOs, cinnamon essential oil and clove essential oil, inhibited completely the growth of *Bacillus subtilis* (Figure 8).



A. B.

Figure 8. Antimicrobial activity of the analysed essential oils: A. - cinnamon (CIO); B. - clove (CLO) on *Bacillus subtilis*

The inhibition diameter zones (diameter of the inhibition halo) values of the analysed essential oils tested on *Bacillus subtilis* are presented in Table 5.

Table 5. Inhibition diameter zones (diameter of the inhibition halo) values of the essential oils tested on *Bacillus subtilis*

Essential oil (µl)	R1 (cm)	R2 (cm)	Mean (cm)	Standard deviation
US 50	9	9	9	0
UC 50	9	9	9	0

CIO - cinnamon essential oil, CLO - clove essential oil

After 48 hours from the inoculation with *Bacillus subtilis* bacterial strain, all tested samples, containing 50 µL of EOs did not exhibit any growth of the microorganism, the inhibition zone (diameter of the inhibition halo) values being of 9 cm (whole surface of the Petri Dish).

The degree of inhibition is presented in Table 6 and it was 100% for both EOs at concentrations of 50 µL.

Table 6. Degree of inhibition of essential oils tested on *Bacillus subtilis*

EOs (µl)	R1 (%)	R2 (%)	Mean (%)	GI (%)
US 50	0	0	0	100
UC 50	0	0	0	100

CIO - cinnamon essential oil, CLO - clove essential oil

Determination of the minimum inhibitory concentration (MIC) of the tested essential oils

The minimum inhibitory concentrations (MIC) of the two essential oils was determined and the results are presented in Table 7.

Table 7. Minimum inhibitory concentration (MIC) of essential oils tested on *Aspergillus brasiliensis*, *Botrytis cinerea*, *Penicillium expansum* and *Bacillus subtilis*

Microorganism	Cinnamon essential oil (CIO)	Clove essential oil (CLO)
	MIC (µl)	
Aspergillus brasiliensis	30	60
Botrytis cinerea	50	50
Penicillium expansum	10	60
Bacillus subtilis	50	50

The minimum inhibitory concentration in the case of cinnamon essential oil was 30 µl for the *Aspergillus brasiliensis*, 50 µl for *Botrytis cinerea* and *Bacillus subtilis* and 10 µl *Penicillium expansum*.

As for the clove essential oil showed that the MIC for *Aspergillus brasiliensis* and *Penicillium expansum* was 60 µl and at 50 µl *Botrytis cinerea and Bacillus subtilis.*

The best results were obtained by the cinnamon essential oil, thus the results obtained are similar to the one in other research articles (El Atki et al., 2019; Gupta et al., 2008).

CONCLUSIONS

There is increasing interest in the development of natural antimicrobial agents and alternative preservation methods in response to consumer demand for natural and clean-label products. Research efforts are focused on exploring new antimicrobial compounds derived from natural sources, as well as innovative preservation technologies that minimize the use of synthetic chemicals. Antimicrobial preservation encompasses a wide range of techniques and strategies aimed at preserving products by inhibiting microorganisms that can cause spoilage or pose a health risk.

Cinnamon essential oil has presented the highest antimicrobial activity for all microroganisms analysed, *Aspergillus brasiliensis*, *Botrytis cinerea*, *Penicillium expansum* and *Bacillus subtilis*.

The degree of inhibition was observed for both cinnamon and clove essential oil, but the best results were obtained for the cinnamon essential oil and concentrations of 10 µl for *Penicillium expansum*. The minimum inhibitory concentration in the case of cinnamon essential oil was between 10 µl and 50 µl for the fungal strains and 50 µl for *Bacillus subtilis*.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

GLIMPSES INTO THE BIODIVERSITY OF SFÂNTU GHEORGHE, TULCEA COUNTY

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Abstract

A field trip carried out in July 2023 reveales some aspects of natural biodiversity in Sfântu Gheorghe, a locality from Tulcea County placed in the Danube Delta Biosphere Reserve. During the observation period, the following fish species were noticed at the shore of the Black Sea: Abramis brama, Callionymus risso, Cyprinus carpio, Esox lucius, Engraulis encrasicolus, Gymnammodytes cicerelus, Ophidion rochei, Pomatoschistus minutus and Trachurus mediterraneus. Of them, only for four species were given data upon the total length (cm) and total weight (grams), taking into account the number of specimens and the quality of preservation. Among the invertebrates gathered from the vegetation, were registered: gastropods (Cepaea hortensis, Helicella sp., Planorbarius corneus), spiders (Agelena sp., Araneus diadematus, Argiope bruennichi, Cheiracanthium sp., Tetragnatha sp.) and insects, mainly from Orthoptera order. In addition, the preliminary study of the coastal flora of the Sfântu Gheorghe indicated a total of 74 plant species, belonging to a number of 37 families. Some of the species are included in the categories of rare species.

Key words*: Black Sea, Danube Delta, Sfântu Gheorghe, species, vegetation.*

INTRODUCTION

The Danube Delta Biosphere Reserve is a protected area at national and international level, and over time it has been designated as a Ramsar site, it has been inscribed on the UNESCO World Heritage list and in two main categories of the Natura 2000 network (Sites of Importance Community - SCI and Special Avifaunistic Protection Areas - SPA).

It was stated that Danube Delta represents a true amphitheater of biodiversity, being characterized by a large number of ecosystems, herbaceous and woody plants species, invertebrate and vertebrate animals (Olaru & Dicu, 2022).

A typical fishermen village from a protected natural area, Sfântu Gheorghe community is focused mainly on fishing and tourism (Bozagievici et al., 2011).

Situated on the coastal strip in the south of the delta, Sfântu Gheorghe supports a large variety of habitats, such as sandy beach, vegetated dunes, dunes with grass cover, salt marshes, meadows, woods and canals with water vegetation (de Vries et al., 2017).

This place from the S-E of Romania is known to have numerous important plant species included in the Red List of the Danube Delta and, considering human activities, the reason for the study was related to the succession of flora and vegetation on the verge of extinction.

Regarding the insect diversity, in the specialist literature, the are few observations upon the *Odonata* fauna from the surroundings of Sfântu Gheorghe Village (de Vries et al., 2017).

Moreover, concerning fisheries, it is known that the community of Sfântu Gheorghe is based mainly on on anadromous migratory fish including Pontic shad, sturgeon, as well as small species such as sprat and anchovy (Marin et al., 2017).

This article aims to contribute to the data about some components of the biodiversity that can be found in the vicinity of the Sfântu Gheorghe, considering the huge potential of this important touristic location of the Danube Delta Biosphere Reserve.

MATERIALS AND METHODS

The data included in this report were collected in July 2023, during a personal field trip that took place in Sfântu Gheorghe, Tulcea County. Most plants and invertebrates were gathered by hand pulling and an entomological net, respectively, mainly from the meadows (collecting points with geographic coordinates 44.893172 N, 29.616194 E; 44.892067 N, 29.611279 E; 44.890950 N, 29.616630 E). Arthropods were preserved and identified according to the protocol mentioned in previous research (Stavrescu-Bedivan et al., 2018).

For the systematic classification of the cormophyte, the *Illustrated Flora of Romania* was used (Ciocârlan, 2000).

The genera within the Kingdom *Plantae*, were noted alphabetically, with the corresponding order and family names given in parentheses, and the vernacular names were given in accordance with online data bases such as *Global Biodiversity Information Facility*, *Plants For A Future* and *World Flora Online*.

After a storm, fish specimens (including still fresh or already dead individuals) could have been captured by hand, near the shore of the Black Sea, identified and photographed.

Regarding the ichthyofauna species mentioned herein, the scientific name and classification were given according to FishBase database (Kottelat & Freyhof, 2007; www.fishbase.se).

Some fish individuals were measured for total length (TL, in cm) and weighed (TW=total body weight, in grams) to the nearest 1.0 mm and 0.1 g, respectively.

RESULTS AND DISCUSSIONS

I. **Fish inventory**

Teleost fish species identified on the shore of the Black Sea during this survey (Figure 1) are: *Abramis brama* (Linnaeus, 1758) (Freshwater bream); *Callionymus risso* (Lesueur, 1814) (Risso's dragonet);*Cyprinus carpio* Linnaeus, 1758 (Common carp); *Engraulis encrasicolus* (Linnaeus, 1758) (European anchovy); *Gymnammodytes cicerelus* (Rafinesque, 1810) (Mediterranean sand eel); *Esox lucius* (Linnaeus, 1758) (Northern pike); *Ophidion rochei* (Müller, 1845) (Roche's snake blenny); *Pomatoschistus minutus* (Pallas, 1770) (Sand goby); *Trachurus mediterraneus* (Steindachner, 1868) (Mediterranean horse mackerel).

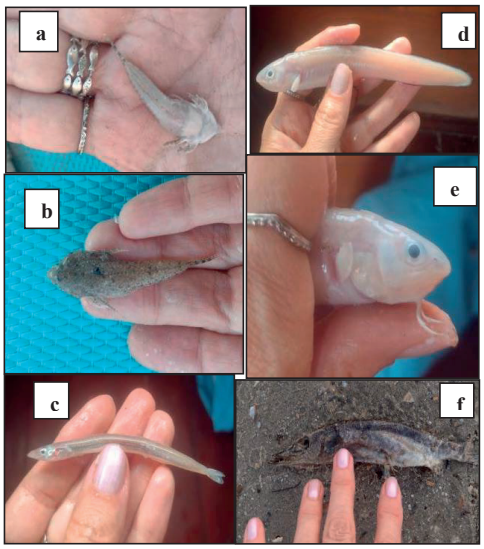


Figure 1. Some fish species identified in Sfântu Gheorghe, Tulcea county (July 2023)*:* a,b. *Callionymus risso*; c. *Gymnammodytes cicerelus*; d, e. *Ophidion rochei*; f. *Esox lucius*

Ophidion rochei (Müller, 1845) (Roche's snake blenny) is an endemic species that lives in the demersal areas of the Mediterranean Sea and the Black Sea. It generally prefers sandy, shallow areas not exceeding 150 m deep (Kéver et al., 2012; Othman et al., 2020). Data on the biology and distribution of the species are scarce, as the fish is nocturnal and hides in the sand during the day. In the specialized literature, *O. rochei* was reported on the Romanian coast from Capul Midia to Mangalia (Niţă et al., 2022), but new data showed that the species was also found in Sfântu Gheoghe (iNaturalist). In July 2023, four individuals belonging to this species were measured (TL ranging from 11 to 14 cm, with TW values from 7.22 to 13.46 g).

Gymnammodytes cicerelus (Rafinesque, 1810) is a common species in the basins of the Mediterranean Sea, the Black Sea, the Sea of Marmara, the Baltic Sea along the European shores of the Atlantic Ocean, but it has also been reported in Morocco, Angola and Senegal (Afonso et al., 2013; Sánchez et al., 2017). The Mediterranean sand eel is a betopelagic fish, which hides in the sand during the day and actively searches for food at night. In Catalonia,

the breeding period has been estimated to extend from November to February, and in mid-December, individuals that have reached their first maturity can be found (Sánchez et al., 2017). Data regarding the reproduction of the species in the Black Sea basin are insufficient. It is assumed that this would end in mid-February-beginning of March (Daban & İşmen, 2020; Niţă et al., 2022).

Callionymus risso (Lesueur, 1814) Risso's dragonet is a demersal fish that inhabits Romanian coastal areas. It is quite widespread in the Mediterranean Sea region, including the Adriatic Sea, the Aegean Sea, the Marmara Sea, as well as the Black Sea. It was very rarely observed in the Eastern Atlantic, more precisely in the west and south of Portugal (iNaturalist; Niţă et al., 2022; Bănăduc et al., 2023). In present survey, four specimens belonging to *C. risso* were measured (TL ranging from 4 to 4.5 cm, TW from 0.6 to 0.96 g).

Trachurus mediterraneus (Steindachner, 1868) Mediterranean horse mackerel is a pelagic and migratory species that occurs in large shoals in the Bay of Biscay region and up to the coastal area of Mauritania, including the Mediterranean Sea. In the region of the Black Sea, the Sea of Azov and the Sea of Marmara, the subspecies *T. mediterraneus ponticus* is found (Kutsyn, 2021). Schools of Mediterranean horse mackerel migrate north to spawn and feed when the water reaches 13-14°C. They appear on the Romanian coast in the spring, between April and May (Yankova, 2011; Yankova, 2013; Păun et al., 2020; Niţă et al., 2022).

Moreover, six specimens of *Engraulis encrasicolus* (with TL ranging from 6 to 9.5 cm and TW from 1.43 to 8.5 g) were registered during personal observations in this field trip.

Engraulis encrasicolus (Linnaeus, 1758) is a small fish (about 10-15 cm average length) of the *Engraulidae* family. It is found in the coastal waters of the eastern Atlantic from Norway to South Africa, being reported from the east coast of Africa. The species is also found in the Mediterranean Sea, the Black Sea and the Sea of Azov, with specimens having crossed the Suez Canal and the Gulf of Suez. In the Black Sea, the european anchovy adapts to fresh and brackish water conditions, characteristic of estuaries and coastal areas. It lives in large groups and migrates according to the season and the availability of food. It is an economically important species (Horton & van der Lingen, 2019). European achovy has been already registered in Sfântu Gheorghe seaside sample station (Nicolae et al., 2018).

Pomatoschistus minutus (Pallas, 1770) Sand goby is a species of the family *Gobiidae* distributed from the north of Scandinavia to the Mediterranean Sea, the neighboring areas of the Eastern Atlantic, the Black Sea, the Baltic Sea. It prefers the benthic way of life, in salty waters, but it can be found in fresh waters, on sandy or muddy bottoms, or even in ecotones where the substrate is hard, at depths of 30-40 m (Salgado et al., 2004; Niţă et al., 2022; Jensen et al., 2023). Two individuals (TL = 4 cm, TW = 1.3 g; TL = 4 cm, TW = 1.42 g) were registered in July 2023 in Sfântu Gheorghe.

Regarding the conservation status of the nine fish species identified on the Romanian coast (according to the IUCN - International Union for Conservation of Nature), for two species there are Data Deficient (DD) for the evaluation of the conservation status - *Ophidion rochei* and *Gymnammodytes cicerelus*, and a species (*Cyprinus carpio*) is classified as Vulnerable (VU). The remaining six species identified on the Romanian coast are Least Concern (LC).

II. **Invertebrate inventory**

During the study period, the following invertebrate taxa were noticed, identified and photographed in the meadows of Sfântu Gheorghe:

Phylum *Mollusca*

Cepaea hortensis (O.F. Müller, 1774) (Gastropoda: *Helicidae*), White-lipped snail;
Helicella spp. (Gastropoda: *Geomitridae*);
Planorbarius corneus (Linnaeus, 1758); (Gastropoda: *Planorbidae*), Great ramshorn.

Phylum *Arthropoda*
Subphylum *Chelicerata*
Agelena sp. (Arachnida: *Araneae*);
Araneus diadematus Clerck, 1758 (Arachnida: *Araneidae*);
Argiope bruennichi (Scopoli, 1772) (Arachnida: *Araneae*) (Figure 2 a);
Cheiracanthium sp. (Arachnida: *Cheiracanthiidae*);
Tetragnatha sp. (Arachnida: *Tetragnathidae*).
Subphylum *Hexapoda*

Aeshna spp. (Odonata: *Aeshnidae*);
Calliptamus italicus (Linnaeus, 1758) (Orthoptera: *Acrididae*), Italian locust;
Carpocoris mediterraneus (Tamanini, 1958) (Hemiptera: *Pentatomidae*), Red shield bug;
Cicadella viridis (Linnaeus, 1758) (Hemiptera: *Cicadellidae*), Green leafhopper;
Coccinella septempunctata (Linnaeus, 1758) (Coleoptera: *Coccinellidae*), Seven spotted lady beetle (Figure 2 b);
Decticus verrucivorus (Linnaeus, 1758) (Orthoptera: *Tettigoniidae*), Wart-biter;
Dociostaurus maroccanus (Thunberg, 1815) (Orthoptera: *Acrididae*), Moroccan locust;
Emmelia trabealis (Scopoli, 1763) (Lepidoptera: *Noctuidae*), Spotted sulphur (Figure 2 c);
Pyrrhocoris apterus (Linnaeus, 1758) (Hemiptera: *Pyrrhocoridae*), Firebug;
Podagrica sp. (Coleoptera: *Chrysomelidae*);
Tettigonia caudata (Charpentier, 1842) (Orthoptera: *Tettigoniidae*), Eastern Green Bush-Cricket;
Tropinota (*Epicometis*) *hirta* (Poda, 1761) (Coleoptera: *Cetoniidae*), Apple Blossom Beetle.

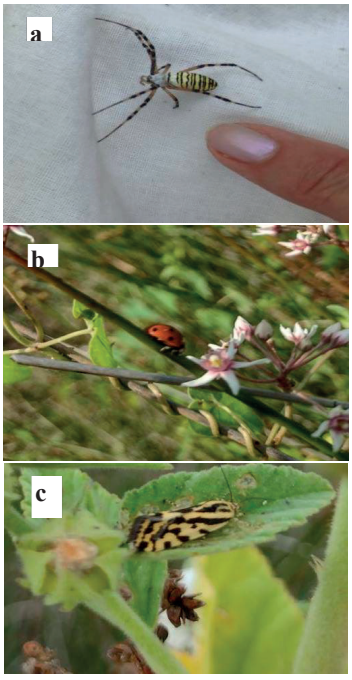


Figure 2. a. *Argiope bruennichi*; b. *Coccinella septempunctata on Cynanchum acutum*; c. *Emmelia trabealis on Althaea officinalis*

III. Floristic inventory

The total identified species during this survey are included in 37 families: *Alismataceae* 1; *Amaranthaceae* 1; *Apiaceae* 3; *Asclepiadaceae* 1; *Asteraceae* 11; *Betulaceae* 1; *Butomaceae* 1; *Chenopodiaceae* 2; *Convolvulaceae* 2 ; *Cyperaceae* 2; *Dipsacaceae* 1; *Eleagnaceae* 2; *Fabaceae* 3; *Geraniaceae* 1; *Hydrocharitaceae* 1; *Juncaceae* 4; *Lamiaceae* 1; *Linaceae* 1; *Lythraceae* 3; *Malvaceae* 1; *Menyanthaceae* 1; *Nymphaeaceae* 2; *Phytolaccaceae* 1; *Plantaginaceae* 2; *Plumbaginaceae* 1; *Poaceae* 6; *Polygonaceae* 3; *Portulacaceae* 1; *Potamogetonaceae* 1; *Ranunculaceae* 2; *Salicaceae* 3; *Salviniaceae* 1; *Scrophulariaceae* 1; *Solanaceae* 1; *Tamaricaceae* 1; *Trapanaceae* 1; *Typhaceae* 1.

A floristic checklist was provided for vascular flora of Sfântu Gheorghe, as follows:
1. *Achillea millefolium* L. (Asterales: *Asteraceae*), Yarrow;
2. *Aeluropus littoralis* (Gouan) Parl. (Poales: *Poaceae*;
3. *Agropogon littoralis* (Sm.) C.E. Hubbard (Poales: *Poaceae*);
4. *Agrostis stolonifera* L. (Poales: *Poaceae*), Creeping bent;
5. *Alisma plantago aquatica* L. (Alismatales: *Alismataceae*), European water plantain;
6. *Alnus glutinosa* (L.) Gaertner (Fagales: *Betulaceae*), Common alder;
7. *Althaea officinalis* L. (Malvales: *Malvaceae*), Marshmallow;
8. *Artemisia annua* L. (Asterales: *Asteraceae*), Sweet sagewort;
9. *Atriplex littoralis* L. (Caryophyllales: *Amaranthaceae*), Grass-leaved orache;
10. *Berula erecta* (Hudson) Coville (Apiales: *Apiaceae*), Cutleaf waterparsnip;
11. *Butomus umbellatus* L. (Alismatales: *Butomaceae*), Flowering rush;
12. *Calystegia sepium* (L.) R.Br. (Solanales: *Convolvulaceae*), Hedge bindweed (Figure 3 a);
13. *Carduus* sp. (Asterales: *Asteraceae*);
14. *Carex* sp. (Cyperales: *Cyperaceae*);
15. *Cichorium intybus* L. (Asterales: *Asteraceae*), Common chicory;
16. *Cirsium alatum* (S.G.Gmelin) Bobrov (Asterales: *Asteraceae*);
17. *Convolvulus persicus* L. (Solanales: *Convolvulaceae*);

18. *Consolida regalis* Gray (Ranunculales: *Ranunculaceae*), Forking larkspur;
19. *Cynanchum acutum* L. (Gentianales: *Asclepiadaceae*);
20. *Cynodon dactylon* (L.) Pers. (Poales: *Poaceae*), Bermuda grass;
21. *Daucus carota* L. (Apiales: *Apiaceae*), Wild carrot;
22. *Dipsacus fullonum* L. (Dipsacales: *Dipsacaceae*), Fuller's teasel;
23. *Eryngium maritimum* L. (Apiales: *Apiaceae*), Sea Holly;
24. *Elaeagnus angustifoli*a L. (Elaeagnales: *Elaeagnaceae*), Russian olive;
25. *Eleocharis palustris* (L.) Roemer et Schultes (Cyperales: *Cyperaceae*), Common spikerush;
26. *Geranium* sp. L. (Geraniales: *Geraniaceae*);
27. *Glycyrrhiza glabra* L. (Fabales: *Fabaceae*), Liquorice;
28. *Hordeum bulbosum* L. (Poales: *Poaceae*), Bulbous barley;
29. *Hydrocharis morsus-ranae* L. (Hydrocharitales: *Hydrocharitaceae*), European frog-bit;
30. *Hyppophae rhamnoides* L. (Rosales: *Elaeagnaceae*), Sea buckthorn;
31. *Juncus conglomeratus* L. (Juncales: *Juncaceae*), Common rush;
32. *Juncus littoralis* C.A.Meyer (Juncales: *Juncaceae*);
33. *Juncus maritimus* Lam. (Juncales: *Juncaceae*), Sea rush;
34. *Juncus subnodulosus* Schrank (Juncales: *Juncaceae*), Blunt-flowered rush;
35. *Lactuca tatarica* (L.) C.A.Meyer (Asterales: *Asteraceae*), Blue lettuce;
36. *Leontodon autumnalis* L. syn. *Scorzoneroides autumnalis* (L.) Moench (Asterales: *Asteraceae*), Fall dandelion;
37. *Leymus sabulosus* (Bieb.) Tzvelev. (Poales: *Poaceae*), Mammoth wild rye;
38. *Limonium bellidifolium* (Gouan) Dumort. - (Plumbaginales: *Plumbaginaceae*), Matted sea-lavender (Figure 3 b);
39. *Linum austriacum* L. (Linales: *Linaceae*), Asian flax;
40. *Lythrum virgatum* L. (Myrtales: *Lythraceae*), Wand loosestrife;
41. *Lythrum tribracteatum* Salzm. ex Spreng. (Myrtales: *Lythraceae*);
42. *Lythrum salicaria* L. (Myrtales: *Lythraceae*), Purple loosestrife;
43. *Nuphar lutea* (L.) Sm. (Nymphaeales: *Nymphaeaceae*), Yellow water lily;
44. *Nymphaea candida* J. Presl (Nymphaeales: *Nymphaeaceae*);
45. *Nymphoides peltata* (S.G.Gmel.) O. Kuntze (Asterales: *Menyanthaceae*), Fringed water lily;
46. *Ononis spinosa* L. (Fabales: *Fabaceae*), Spiny restharrow (Figure 3 c);
47. *Periploca graeca* L. (Gentianales: *Asclepiadaceae*), Silk vine;
48. *Petasites spurius* (Retz.) Reichenb. (Asterales: *Asteraceae*);
49. *Plantago lanceolata* L. (Plantaginales: *Plantaginaceae*), Buckhorn;
50. *Plantago maritima* L. (Plantaginales: *Plantaginaceae*), Sea plantain;
51. *Phytolacca americana* L. (Caryophyllales: *Phytolaccaceae*), American Pokeweed;
52. *Polygonum graminifolium* Wierzb. ex Heuff. (Polygonales: *Polygonaceae*);
53. *Polygonum aviculare* L. (Polygonales: *Polygonaceae*), Prostrate knotweed;
54. *Populus nigra* L. (Salicales: *Salicaceae*), Black poplar;
55. *Portulaca oleracea* L. (Caryophyllales: *Portulacaceae*), Common purslane;
56. *Potamogeton pusillus* L. (Najadales: *Potamogetonaceae*), Small pondweed;
57. *Pulicaria dysenterica* (L.) Bernh. (Asterales: *Asteraceae*), Meadow false fleabane;
58. *Ranunculus circinatus* Sibth. (Ranunculales: *Ranunculaceae*), Fan-leaved water-crowfoot;
59. *Rumex aquaticus* L. (Polygonales: *Polygonaceae*), Red dock;
60. *Salicornia europaea* L. (Caryophyllales: *Chenopodiaceae*), Glasswort;
61. *Salix fragilis* L. (Salicales: *Salicaceae*), Crack willow;
62. *Salix viminalis* L. (Malpighiales: *Salicaceae*), Basket willow;
63. *Salvinia natans* (L.) All. (Salviniales: *Salviniaceae*), Floating watermoss;
64. *Scolymus hispanicus* L. (Asterales: *Asteraceae*), Common golden thistle (Figure 3 d);
65. *Senecio vulgaris* L. (Asterales: *Asteraceae*), Common fireweed;
66. *Solanum dulcamara* L. (Solanales: *Solanaceae*), Climbing nightshade;
67. *Sonchus arvensis* L. (Asterales: *Asteraceae*), Field milk thistle;
68. *Stachys* sp. (Lamiales: *Lamiaceae*);

69. *Suaeda maritima* (L.) Dumort. (Caryophyllales: *Chenopodiaceae*), Seablite;
70. *Tamarix ramosissima* Ledeb. (Tamaricales: *Tamaricaceae*), Saltcedar;
71. *Trapa natans* L. (Myrtales: *Trapanaceae*), Water chestnut;
72. *Trifolium angulatum* Waldst.et.Kit. (Fabales: *Fabaceae*), Angled clover;
73. *Typha latifolia* L. (Typhales: *Typhaceae*), Broadleaf cattail;
74. *Verbascum banaticum* Schrad. (Scrophulariales: *Scrophulariaceae*).

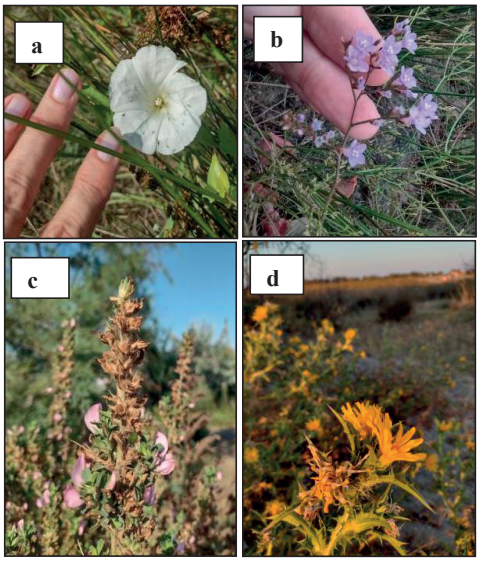


Figure 3. Some plant species identified in Sfântu Gheorghe, Tulcea county (July 2023):
a. *Calystegia sepium*; b. *Limonium bellidifolium*; c. *Ononis spinosa*; d. *Scolymus hispanicus*

Some of the observed species are included in the Red List of Romania in the following categories: R - rare; V - vulnerable.
Cirsium alatum R, *Convolvulus persicus* - R, *Eryngium maritimum* - R, *Glycyrrhiza glabra* L. - R, *Hordeum bulbosum* - R, *Limonium bellidifolium* - R, *Lythrum tribracteatum* - R, *Nymphaea candida* - R, *Petasites spurius* - R, *Polygonum graminifolium* - R, *Ranunculus circinatus* - R, *Scolymus hispanicus* - R, *Trapa natans* - V, *Trifolium angulatum* - R.
The associations of *Juncetum maritimi*, *Suaedetum maritimae* or *Salicornietum europaeae* were included by Gâștescu (2021) into the meadows on low marine levees with sandy and halophile vegetation, a type of natural ecosystem described in the Danube Delta Biospehere Reserve.

CONCLUSIONS

On the basis of observations during the field trip placed in the area of Sfântu Gheorghe, Tulcea county, in July 2023, 74 plant species belonging to a number of 37 families were determined. The families *Asteraceae* (9) and *Poaceae* (6) comprise the most species in the area.
Among the invertebrates collected from the vegetation of meadows, are worth mentioning gastropods, spiders and insects - maninly *Orthoptera* species.
Concerning the ichthyofauna associated with the biodiveristy of Sfântu Gheorghe, some biometric measurements were registered, for *Callionymus risso, Engraulis encrasicolus, Ophidion rochei* and *Pomatoschistus minutus* fish species.
Since IUCN assessed *Ophidion rochei* and *Gymnammodytes cicerelus* as species classified into Data Deficient (DD) category, data reported herein are important for conservation science.
Sfântu Gheorghe village from the Danube Delta is of particular biological importance, due to its position in a unique and extremely diverse ecosystem. The Danube Delta is one of the largest and best preserved wetlands in Europe and a real refuge for a variety of plant and animal species, some of them rare or endangered.
Knowing the fauna and flora in the area of Sfântu Gheorghe is of major importance from several perspectives: ecological, economic and conservation. This contributes to maintaining the balance of ecosystems, to supporting the local community through sustainable economic activities, as well as to the protection of endangered species.

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Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

SOME AGROBIOLOGICAL FEATURES AND QUALITY INDICES OF THE BIOMASS OF *Panicum virgatum* and *Panicum miliaceum* GROWN UNDER THE CONDITIONS OF THE REPUBLIC OF MOLDOVA

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Abstract

This research was conducted to determine the productivity of switchgrass - Panicum virgatum and common millet - Panicum miliaceum, grown in monoculture in the experimental plot of the NBGI Chişinău. It was determined that the dry matter yield and its biochemical composition varied among the Panicum species: 8.2-14.1 kg/m^2 with 61-132/kg CP, 352- 419 g/kg CF, 82-101 g/kg ash, 372-433 g/kg ADF, 608-692 g/kg NDF, 39-59 g/kg ADL, 124-127 g/kg TSS, 333-381 g/kg Cel, 236-259 g/kg HC and the nutritive and energy values of the fresh mass fodder reached 55.2-59.9 % DDM, 9.04-9.73 MJ/kg ME and 5.05-5.75 MJ/kg NEl. The hay prepared from the studied Panicum species contained 56-132 g/kg CP, 79-104 g/kg ash, 348-384 g/kg Cel, 243-272 g/kg HC with 54.5-58.8 % DDM, 8.94-9.57 MJ/kg ME and 4.96-5.59 MJ/kg NEl. The prepared silages had pH index = 4.06-4.14, 30.6-32.5 g/kg organic acids with 88.0-93.5% lactic acid. We found that the biochemical methane potential of the studied substrates varied from 302 to 364 l/kg ODM. Panicum virgatum and Panicum miliaceum may be used as forages for livestock and as feedstock for renewable energy production in Moldova.

Key words*: agrobiological features, biomass, biochemical composition, forages, methane potential, Panicum miliaceum, Panicum virgatum.*

INTRODUCTION

Panicum L. is a genus in the family Poaceae, with nearly 450 species of annual or perennial grasses, occurring in areas with tropical and warm temperate climate. Many representatives of this genus are popular agricultural and horticultural crops, due to their economic significance.

Common millet or proso millet, *Panicum miliaceum* L. is one of the world's oldest cultivated plants as grains and forage crops, which has also been cultivated in our region. *Panicum miliaceum* is a summer annual plant, the stems are light green, erect, sometimes branched at the base, growing about 0.5-1.5 m tall, with bright green leaves, compact panicle, the grains are round, about 3 mm long and 2 mm wide, enclosed in a smooth hull, which is typically white or creamy-white, yellow, or red, but also may be gray, brown or black. Plants have shallow, fibrous root systems and produce few tillers. It is a short growing, summer season crop (60 to 100 days) with unique agronomic properties such as high tolerance to heat and drought conditions. *Panicum miliaceum* could be a viable alternative to main summer forages in areas where cultivation of corn or Sudan grass is restricted because of a longer growing season or poor agricultural conditions. The *Panicum miliaceum* have recently been subjects of research around the world (Jahansouz et al., 2014; Habiyaremye et al., 2017; Bhat et al., 2019; Ventura et al., 2020; Wei et al., 2021; Turkestani et al., 2023; Wimalasiri et al., 2023; Petcu et al., 2024).

Switchgrass, *Panicum virgatum* L. is native to North America and is the most abundant in the Great Plains and eastern states of US. It is a warm-season perennial and rhizomatous plant, sometimes forming a knotty crown. Stems are green or purplish, erect or decumbent, 40-300 cm tall and 3-5 mm thick, forming dense clumps. The ligules are 2-6 mm; the blades 30-60 cm long and 2-15 mm wide, flat, erect; the adaxial surfaces sometimes densely pubescent; the margins scabrous, with a prominent midrib. The inflorescences are open panicles, 10-55 cm long and 4-20 cm wide, the primary branches thin and straight; the spikelets are 2.5-8 mm long

and 1.2-2.5 mm wide, narrowly lanceoloid, turgid to slightly laterally compressed, glabrous, acuminate. The seeds are 3-6 mm long and 1.2 to 1.5 mm wide and are developed from a single-flowered spikelet. Switchgrass generally produces seeds abundantly, the seed yield in cultivated stands is on average 500-700 kg/ha. Switchgrass reproduces both by seeds and rhizomes. The rhizomes are responsible for the vegetative expansion, and some rhizomes of sod-forming ecotypes may extend to lengths of 0.3-0.6 m, the roots may reach soil depths of 3 m or more. This perennial grass grows on a wide variety of soil types and textures, land conditions, pH from 4.5 to 7.6. It is tolerant of moderate soil salinity and acidity, used for a variety of revegetation and erosion control purposes. Switchgrass is suitable for growth on marginal land and requires relatively modest amounts of chemical fertilizers. The method of establishing switchgrass stands is planting seeds in a properly prepared soil, after the soil has warmed well during the spring, using highly germinable seeds and planting 0.6-1.2 cm deep, or up to 2 cm deep in sandy soils, packing or firming the soil both before and after seeding. Switchgrass in North American an important livestock forage, used primarily for warm-season pasture and hay (Griffin & Jung, 1983; Guretzky et al., 2011; Belanger et al., 2012; Jefferson & McCaughey, 2012; Davis et al., 2018). It has also been introduced as a forages plants to other parts of the world (Ferri et al., 2000; Pilat et al., 2007; Eliş & Özyazıcı, 2019). *Panicum virgatum* has received much attention in recent years and has been a focus of bioenergy research for over a decade, in several countries (Massé et al., 2010; 2011; Rakhmetov et al., 2014a.; 2014 b; Niu et al., 2015; Seepaul et al., 2016; Baute et al., 2018; Kieffer et al., 2023; Happs et al., 2024).

This research was conducted to determine the quality indices of the green mass, prepared silage and hay from *Panicum miliaceum* and *Panicum virgatum* as forages for livestock, as well as feedstock in biogas reactors for the production of biomethane as renewable energy.

MATERIALS AND METHODS

The common millet - *Panicum miliaceum* cultivar 'Soiuz' created at the at the "Selectia" Research Institute of Field Crops Bălți, Republic of Moldova, and the introduced taxa of switchgrass - *Panicum virgatum* grown in the experimental plot of the National Botanical Garden (Institute) "Alexandru Ciubotaru", Chişinău, served as subjects of the research.

The *Panicum miliaceum* plant samples were collected in the flowering stage, while the *Panicum virgatum* plants were mown in middle flowering stage, in the second year of life. The harvested plants were chopped into 1.5-2.0 cm small pieces, with a laboratory forage chopper, the dry matter content was detected by drying samples up to constant weight at 105°C. The silage was prepared from chopped green mass, compressed in well-sealed glass containers, stored at ambient temperature (18-20°C). After 45 days, the containers were opened, and the sensorial and fermentation indices of the conserved forage were determined according to standard laboratory procedures - the Moldavian standard SM 108*. The prepared hay was dried directly in the field. For biochemical analysis, the plant samples were dried in a forced air oven at 60°C, milled in a beater mill equipped with a sieve with diameter of openings of 1 mm and some assessments of the main biochemical parameters: crude protein (CP), ash, acid detergent fibre (ADF), neutral detergent fibre (NDF), acid detergent lignin (ADL), total soluble sugars (TSS), digestible dry matter (DDM), digestible organic matter (DOM) have been determined by near infrared spectroscopy (NIRS) technique PERTEN DA 7200. The concentration of hemicellulose (HC), cellulose (Cel), digestible energy (DE), metabolizable energy (ME), net energy for lactation (NEl) and relative feed value (RFV) were calculated according to standard procedures.

The carbon content of the substrates was determined using an empirical equation according to Badger et al. (1979). The biochemical methane potential was calculated according to the equations of Dandikas et al. (2015).

RESULTS AND DISCUSSIONS

Analysing some agrobiological peculiarities of the studied *Panicum* species (Table 1), we would like to mention that in the flowering stage *Panicum miliaceum* plants reach 84 cm in

height, but *Panicum virgatum* - 127 cm. The biomass productivity of *Panicum miliaceum* was 3.59 kg/m^2 fresh mass or 0.82 kg/m^2 dry matter, while - of *Panicum virgatum* 3.85 kg/m^2 fresh mass or 1.41 kg/m^2 dry matter. The content of leaves and panicles in *Panicum miliaceum* fodder was much higher, but the dry matter content lower than in *Panicum virgatum.*

Analysing the results of the fresh mass quality of the studied *Panicum* species (Table 2), we found that the dry matter of the harvested whole plants contained 61-132 g/kg CP, 352-419 g/kg CF, 82-101 g/kg ash, 372-433 g/kg ADF, 608-692 g/kg NDF, 39-59 g/kg ADL,124-127 g/kg TSS, 333-381 g/kg Cel, 236-259 g/kg HC. The *Panicum virgatum* green fodder is characterized by higher cell wall content and lower concentration of crude protein and minerals than the fodders from *Panicum miliaceum*. The concentration of total soluble sugars is very high and does not differ particularly between the fodders from the studied *Panicum* species. The nutritive and energy values of the fresh mass from the studied *Panicum* species reached 55.2-59.9% DDM, 11.01-11.86 MJ/kg DE, 9.04-9.73 MJ/kg ME and 5.05-5.75 MJ/kg NEl. The high amount of cell wall content in switchgrass plants contributed to the reduction of the dry matter digestibility, relative feed value, metabolizable energy and net energy for lactation. The green forage from *Panicum miliaceum* plants had optimal concentration of crude protein, fibres, acid detergent lignin digestibility and energy concentration.

Table 1. Some agrobiological peculiarities of the studied *Panicum* species

Species	Plant height, cm	Stem, g		Leaf + panicle, g		Total weight of a shoot, g		Yield, kg/m^2		Content of leaves and panicles in fodder, %
		fresh mass	dry matter	fresh mass	dry matter	fresh mass	dry matter	fresh mass	dry matter	
Panicum miliaceum	84	14.7	3.2	14.6	3.5	29.3	6.7	3.59	0.82	52.2
Panicum virgatum	127	4.1	1.5	3.0	1.1	7.1	2.6	3.85	1.41	42.0

Table 2. The biochemical composition and the nutritive value of the green mass harvested from *Panicum* species

Indices	*Panicum virgatum*	*Panicum miliaceum*
Crude protein, g/kg DM	61	132
Crude fibre, g/kg DM	419	352
Minerals, g/kg DM	82	101
Acid detergent fibre, g/kg DM	433	372
Neutral detergent fibre, g/kg DM	692	608
Acid detergent lignin, g/kg DM	52	39
Cellulose, g/kg DM	381	333
Hemicellulose, g/kg DM	259	236
Total soluble sugars	127	124
Digestible dry matter, g/kg DM	552	599
Relative feed value	74	92
Digestible energy, MJ/ kg DM	11.01	11.86
Metabolizable energy, MJ/ kg DM	9.04	9.73
Net energy for lactation, MJ/ kg DM	5.05	5.75

Different results regarding the nutrient content of the harvested mass from *Panicum* species are given in the specialized literature. According to Griffin & Jung (1983), the forage quality of leaf mass of *Panicum virgatum* harvested in early heading stage was 8.5% CP, 67.1% NDF, 4.7% lignin and 58.8% IVDMD, while stem mass - 3.8% CP, 77.7% NDF, 8.3% lignin and 47.2% IVDMD. Burlacu et al. (2002) mentioned that the *Panicum miliaceum* green mass contained 230 g/kg DM with 8.7% ash, 14.0% CP, 4.7% EE, 27.1% CF, 45.5% NFE and 18.5 MJ/kg GE. Dien et al. (2006) reported that switchgrass plant material harvested in pre-boot stage had 6.5% CP, 1.0% EE, 8.9% ash, 66.9% NDF, 1.2% ADL, 33.7% Cel, 31.8% HC and 18.22 MJ/kg GE, while plant harvested in anthesis stage 3.2% CP, 1.0% EE, 5.7% ash, 66.9% NDF, 2.3% ADL, 34.0% Cel, 30.1% HC and 18.61 MJ/kg GE. Pilat et al. (2007) reported

that the green forage dry matter from switch grass contained 274.9 g/kg DM with 92.84% OM, 10.46% CP, 1.48% CT, 32.60% CF, 72.33% NDF, 39.23% ADF, 33.1% HC and 48.3 % NFE. Svirskis (2009) found that, under the climatic conditions of Lithuania, the dry matter of the studied *Panicum miliaceum* cultivars contained 12.6-19.6% CP, 28.0-35.6% CF, 56.6-65.3% NDF, 25.3-33.1% ADF, 4.56-11.39% WSC and 48.4-65.3% DMD. Guretzky et al. (2011) remarked that switch grass had 8.5-10.5 N g/kg, 261-381 g/kg CF, 426-455 g/kg ADF, 740-752 g/kg NDF, 411-492 g/kg IVDMD. Belanger et al. (2012) remarked that *Panicum virgatum* biomass harvested on four different dates contained 40.2-117.7 g/kg TSS, 297-415 g/kg ADF, 551-705 g/kg NDF, 582-843 g/kg IVTD. Jefferson & McCaughey (2012) mentioned that the forage dry matter from *Panicum virgatum* cultivars had 53.7-64.7% NDF, 28.2-33.0% Cel, 26.2-30.2% HC, with 44.3-61.2% OMD. Zhang et al. (2010) mentioned that, in northern China, dry biomass from proso millet contained 12.8% CP, 73.3% NDF, 40.2% ADF, from Sudan grass - 12.0% CP, 62.7% NDF, 38.4% ADF and maize fodder dry matter - 10.8% CP, 66.1% NDF and 42.8% ADF. Avetisyan (2013) reported that the quality indices of the green mass from proso millet were 200 g/kg dry matter with 9.8% CP, 38.9% CF, 6.2% sugar, 9.7 MJ/kg ME. Mohajer et al. (2013) mentioned that the dry matter of the studied common millet cultivars contained 9.15-10.78% CP, 43.45-44.84% CF, 5.58-7.16% WSC, 28.17-29.50% ADF and 64.74-65.90% DMD. Jahansouz et al. (2014) reported that the quality indices of green mass from *Panicum miliaceum* were 10.91% CP, 76.34% NDF, 50.82% ADF, 6.29% WSC, 49.31% DMD, 0.98 Mcal/kg NEl and RFV = 60.1. Seepaul et al. (2016) remarked that switchgrass whole plant harvested in the June-July period had 3.5-4.6% CP, 6.5-7.7% ash, 73.9-80.5% NDF, 40.4-45.9% ADF, 4.6-7.9% ADL, 33.5-35.1% Cel, 32.9-40.9% HC, 553-589 g/kg IVDMD. Tran (2016) remarked that proso millet forage was characterized by the following quality indices 7.9% ash, 10.1% CP, 73.7% NDF, 36.0% ADF, 4.5% lignin, 3.6 g/kg Ca, 1.6 g/kg P, 58.5% DOM. Wei et al. (2021) reported that proso millet green mass had 303.4 g/kg dry matter with 6.31% CP, 32.6% ADF, 607.2% NDF and 649.5 g/kg IVDMD. Park et al. (2022) stated that *Panicum miliaceum* forage had 5.7-10.4% CP, 63.1-65.4% NDF, 36.3-37.0% ADF, 59.7-60.2% TDN and RFV = 86-89, but from *Panicum coloratum* 12.0-12.4% CP, 57.8-62.2% NDF, 30.3-31.7% ADF, 63.9-64.9% TDN and RFV = 95-105, respectively. Van Die & Entz (2022) reported that the proso millet green forage dry matter had 8.0-10.0% CP and 64.8% TDN. Kieffer et al. (2023) remarked that *Panicum virgatum* biomass was characterized by the following quality indices: 3.65% ash, 6.72% CP, 74.60% NDF, 42.88% ADF, 4.06% lignin, 38.97% Cel, 31.73% HC, RFV=69.84, 3.76 g/kg Ca, 1.75 g/kg P. In our previous studies (Țîței, 2023) we found that nutrient content and forage value of proso millet plants was 12.3% CP, 8.4% ash, 38.0.6% ADF, 63.0% NDF, 3.9% ADL, 12.1% TSS, 34.1% Cel, 25.0% HC, 593 g/kg DDM, RFV= 88, 11.75 MJ/kg DE, 9.65 MJ/kg ME and 5.65 MJ/kg NEl.

Table 3. The biochemical composition and the nutritive value of the hay from *Panicum* species

Indices	*Panicum virgatum*	*Panicum miliaceum*
Crude protein, g/kg DM	56	132
Crude fibre, g/kg DM	420	368
Minerals, g/kg DM	79	104
Acid detergent fibre, g/kg DM	441	386
Neutral detergent fibre, g/kg DM	713	629
Acid detergent lignin, g/kg DM	57	38
Cellulose, g/kg DM	384	348
Hemicellulose, g/kg DM	272	243
Total soluble sugars	109	93
Digestible dry matter, g/kg DM	545	588
Relative feed value	71	87
Digestible energy, MJ/ kg DM	10.89	11.66
Metabolizable energy, MJ/ kg DM	8.94	9.57
Net energy for lactation, MJ/ kg DM	4.96	5.59

Hay is the most commonly used forage for ruminant animals, horses, rabbits etc. The quality indices of the hay prepared from the studied *Panicum* species are shown in Table 3. We found that the hay prepared from the studied *Panicum* species contained 56-132 g/kg CP, 79-104 g/kg ash, 348-384 g/kg Cel, 243-272 g/kg HC. The nutritive and energy value of the prepared hays were 54.5-58.8% DDM, 8.94-9.57 MJ/kg ME and 4.96-5.59 MJ/kg NEl. During the hay making process, the concentration of structural carbohydrates increased and the total soluble sugar content decreased. As compared with the *Panicum miliaceum* hay, the *Panicum virgatum* hay is characterized by lower amount of crude protein, minerals, but had high concentration of cell wall fractions (NDF, ADF, ADL) and total soluble sugars, which resulted in the reduction of digestibility, relative feed value and energy concentration.

Several literature sources described the quality indices of the hay prepared from *Panicum* species. According to Ferri et al. (2000) mentioned that *Panicum virgatum* hay contained 749 g/kg DM with 1.3% CP, 5.3% ash, 85.9% NDF, 54.3% ADF, 5.6% Klason lignin and 33.6% *in vivo* dry matter apparent digestibility. Burlacu et al. (2002) mentioned that *Panicum miliaceum* hay had 850 g/kg dry matter with 12.5% ash, 5.8% CP, 2.1% EE, 33.5% CF, 51.2% NFE and 17.9 MJ/kg GE. Tan et al. (2016) mentioned that *Panicum miliaceum* hay, without nitrogen fertilization, had 12.39% CP, 58.40% NDF, 36.05% ADF, but in the variants when nitrogen fertilizers were applied: 13.16-14.24% CP, 58.09-60.02% NDF, 35.73-36.10% ADF, respectively. Tran (2017) reported that the concentration of nutrients and energy in proso millet hay were 7.9% ash, 12.5% CP, 2.5% EE, 33.9% CF, 72.3% NDF, 39.9% ADF, 5.9% lignin, 6.6% ash, 59.3% DOM, 18.9 MJ/kg GE, 10.7 MJ/kg DE, 8.6 MJ/kg ME. Davis et al. (2018) reported that the hay prepared from *Panicum virgatum* cultivars, harvested at different stages of maturity, had 901-912 g/kg DM with 4.8-13.5% CP, 57.1-65.1% NDF, 29.2-36.7% ADF, 51.50-72.6% apparent DMD. Dağtekin et al. (2020) found that the hays from 11 genotypes of proso millet contained 16.3-20.5% CP, 28.7-36.3% ADF, 60.0-70.6% NDF, 0.373-0.434% P, 0.58 - 0.824% Ca, 0.270-0.393% Mg, 3.17-3.96% K. Stybayev et al. (2023) reported that the chemical composition of the proso millet hay in the variant without cover crop was 9.07% CP, 2.76% EE, 30.32% CF, 3.32% sugar, 9.25% ash, 1.11% Ca, 0.23% P, but in the variant with cover crop, the proso millet hay contained 9.62% CP, 3.74% EE, 31.32% CF, 10.20% sugar, 4.23% ash, 2.1% Ca, 0.33% P, respectively. As a result of our previous studies (Țîței, 2024) we found that the nutrient content of *Panicum miliaceum* hay was: 13.40% CP, 2.37% EE, 32.22% CF, 42.29% NFE, 9.72% ash, 0.32% Ca and 0.27% P, 18.02 MJ/kg GE. Forage conservation provides a more uniform level of high quality forage for ruminant livestock throughout the year. Silage fodder is a key element for good farm animal feeding. When opening the glass containers with ensiled mass from the studied *Panicum* species, there was no gas or juice leakage from the preserved mass. As for the organoleptic properties, the prepared silage from *Panicum miliaceum* had yellowish-green stems and leaves with pleasant smell like pickled apples, while silage from *Panicum virgatum* had had homogeneous agreeable olive colour with pleasant smell specific to grass silage. The texture of the ensiled mass from *Panicum* species was preserved well, without mold and mucus. The fermentation profile, the biochemical composition and nutritional value of silage from the studied *Panicum* plants is shown in Table 4. The prepared silages were characterized by pH = 4.06-4.14, 30.6-32.5 g/kg organic acids, 28.5-28.6 g/kg lactic acid, 2.0-3.6 g/kg acetic acid, 0.1-0.3 g/kg butyric acid, the most organic acids were in fixed form, lactic acids constituted 88.0-93.1% of total organic acids. The obtained silages met the standard SM 108 for the 1-st class quality. In the *Panicum virgatum* silage, the concentration of acetic and butyric acids was lower as compared with the silage prepared from *Panicum miliaceum*. The quality indices of silage dry matter were: 6.2-11.6% CP, 8.2-10.3% ash, 34.7-41.4% CF, 36.0-42.3% ADF, 62.0-6.57% NDF, 2.6-4.3 % ADL, 33.4-38.0% Cel, 23.4-26.0% HC, 559-609 g/kg DDM, RFV = 79-91, 11.14-12.03 MJ/kg DE, 9.15-9.87 MJ/kg ME and 5.17-5.89 MJ/kg NEl. It was found that, during the process of ensiling, the concentrations of acid detergent lignin and total soluble sugars decreased. In comparison with the initial green mass, in common millet silage the level of crude protein, crude fibre, acid detergent fibre decreased, the cellulose content

did not change and the concentration of hemicellulose increased substantially, which had a positive impact on the digestibility and energy value. In the switch grass silage crude protein, the cellulose and ash content did not change essentially, the hemicellulose content decreased, the dry matter digestibility and energy concentration differed considerably as compared with the initial green mass.

Table 4. The quality indices of the silage from *Panicum* species

Indices	*Panicum virgatum*	*Panicum miliaceum*
pH index	4.14	4.06
Organic acids, g/kg DM	30.6	32.5
Free acetic acid, g/kg DM	0.2	2.0
Free butyric acid, g/kg DM	0.1	0
Free lactic acid, g/kg DM	4.1	10.6
Fixed acetic acid, g/kg DM	1.8	1.6
Fixed butyric acid, g/kg DM	0	0.3
Fixed lactic acid, g/kg DM	24.4	18.0
Total acetic acid, g/kg DM	2.0	3.6
Total butyric acid, g/kg DM	0.1	0.3
Total lactic acid, g/kg DM	28.5	28.6
Acetic acid, % of organic acids	6.53	11.09
Butyric acid, % of organic acids	0.33	0.91
Lactic acid, % of organic acids	93.14	88.00
Crude protein, g/kg DM	62	116
Crude fibre, g/kg DM	414	347
Minerals, g/kg DM	82	103
Acid detergent fibre, g/kg DM	423	360
Neutral detergent fibre, g/kg DM	657	620
Acid detergent lignin, g/kg DM	43	26
Cellulose, g/kg DM	380	334
Hemicellulose, g/kg DM	234	260
Total soluble sugars	21	121
Digestible dry matter, g/kg DM	559	609
Relative feed value	79	91
Digestible energy, MJ/ kg DM	11.14	12.03
Metabolizable energy, MJ/ kg DM	9.15	9.87
Net energy for lactation, MJ/ kg DM	5.17	5.89

Some authors mentioned various findings about the quality of the silage prepared from *Panicum* species. Pilat et al. (2007) reported that silage forage matter from switch grass had pH = 4.57, 1.19% lactic acid, 0.71% acetic acid, 0.08% butyric acid, contained 240.4 g/kg DM with 91.61% OM, 11.84 % CP, 2.99% CT, 33.91% CF, 74.57% NDF, 43.22% ADF, 31.35%HC, 42.87%NFE. Belanger et al. (2012) mentioned that the prepared silages from *Panicum virgatum* were characterized by: pH = 4.0-4.3, 3.99-5.80% lactic acid, 0.26-0.77% acetic acid, 0.79-1.81% N. Maksimova et al. (2019) found that the quality indices of proso millet silage were pH = 5.0, 0.55 feed units/kg dry matter, 39.0 g/kg digestible protein, and 8.21 MJ/kg exchange energy and 72.5 g digestible proteins/ feeding unit. Eliş & Özyazıcı (2019) stated that the silage obtained from switchgrass cultivars have pH = 3.84-4.73, 390-510 g/kg dry matter with 1.78-2.35% lactic acid, 0.35-1.55% acetic acid, 0.05-0.33% butyric acid, 3.76-5.33% CP, 70.96-75.41% NDF, 39.30-41.73% ADF, 18.75% TSS, 35.13% HC, 10.44% ash. Nematpour et al. (2020) mentioned that the proso millet silage had pH = 4.68, 1.68% acetic acid, 265 g/kg dry matter with 8.14% ash. 16.1% CP, 55.4% NDF, 27.3% ADF, 65.2% DDM, RFV=114 and 1.58 Mcal/kg NEl. Wei et al. (2021) found that the chemical composition and nutritive value of proso millet was pH = 4.35, 4.01% lactic acid, 1.41% acetic acid, 0.23% butyric acid, 266 g/kg dry matter with 5.66% CP, 35.8% ADF, 62.4% NDF, 58.0% IVDMD, 605.5 g/kg TDN and RFV = 91; corn silage pH = 3.86, 6.15% lactic acid, 4.86% acetic acid, 263 g/kg dry matter, 5.20% CP, 26.52% ADF, 46.51% NDF, 68.46% IVDMD, 692.8 g/kg TDN and RFV = 137; but sorghum-Sudan grass silage had pH = 4.07, 7.67% lactic acid, 4.52% acetic acid, 175.4 g/kg

dry matter, 4.69% CP, 42.80% ADF, 65.48% NDF, 54.76% IVDMD, 547.6 g/kg TDN and RFV = 78, respectively. In our previous research (Ţîţei, 2023; 2024), the quality indices of common millet silages were as follows: 11.0-13.3% CP, 4.0% EE 36.7-37.0% CF, 37.1% ADF, 63.0% NDF, 2.0% ADL, 6.8% TSS, 35.1% Cel, 23.7% HC, 9.7-11.5% ash, 0.31% Ca, 0.27% P, 600 g/kg DDM, RFV=92, 18.38 MJ/kg GE, 11.87 MJ/kg DE, 9.75 MJ/kg ME, 5.76 MJ/kg NEl. Turkestani et al. (2023) mentioned that proso millet silages had pH = 4.5-4.8 and contained 343-395 g/kg dry matter with 9.0-12.1% CP, 28.8% ADF, 47.4-50.5% NDF, 3.38-3.56% ADL, 593-633 g/kg DMD.

Increasing biomass usage leads to the reduction of greenhouse gas emissions, as compared with the use of fossil fuels. In recent years, the considerations for the use of *Poaceae* species for bioenergy have increased considerably, it can be used as biomass feedstock for the production solid fuel, lignocellulosic bioethanol, synthetic natural gas or synthetic biofuels and, in particular, for biogas production. The C_4 grasses are considered as a potential feedstock for biogas production, due to their low water consumption as compared with other crops, and the fact that they can be cultivated in non-arable lands, avoiding the direct competition with food crops. Biogas is a product of anaerobic fermentation of organic products. Anaerobic fermentation is a labile process whose normal course depends on many factors, including the content of lignin in the substrate. Among the fuels from plant biomass, biogas is of great importance and can successfully replace fossil fuels to obtain electric power and heat. The quantities of biogas and the methane content depend mainly on carbohydrates, fats and proteins, the biodegradability and ratio of carbon and nitrogen (C/N) from the substrates. The results of the determination of the quality indices of prepared substrates from the studied *Panicum* species and its biochemical biomethane production potential are presented in Table 5.

Table 5. The biochemical biomethane production potential of the investigated substrates from *Panicum* species

Indices	*Panicum virgatum*			*Panicum miliaceum*		
	green mass	silage	hay	green mass	silage	hay
Crude protein, g/kg DM	61.00	62.00	56.00	132.00	116.00	132.00
Nitrogen, g/kg DM	9.76	9.92	8.96	21.12	18.56	21.12
Ash, g/kg DM	82.00	82.00	79.00	101.00	103.00	104.00
Carbon, g/kg DM	510.00	510.00	511.67	499.44	498.33	497.78
Ratio carbon/nitrogen	52.25	51.14	57.11	23.67	26.85	23.57
Acid detergent lignin, g/kg DM	52.00	43.00	57.00	39.00	26.00	38.00
Hemicellulose, g/kg DM	259.00	234.00	272.00	236.00	260.00	243.00
Biomethane potential, L/kg VS	311	326	302	346	365	348
Biomethane potential, L/kg DM	285	299	278	311	327	312

It is a commonly known fact that methanogenic bacteria need a suitable ratio of carbon to nitrogen for their metabolic processes, ratios higher than 30:1 were found to be unsuitable for optimal digestion, and ratios lower than 10:1 were found to be inhibitory, because of low pH, poor buffering capacity and high concentrations of ammonia in the substrate. The C/N ratio is favorable in *Panicum miliaceum* green mass, silage and hay substrates. In *Panicum virgatum* substrates there is a very high carbon to nitrogen ratio - C/N = 52-57, which may have a negative impact on the methanogenesis processes, that is why it is necessary to use mixed substrates with other plants. As previously mentioned, in the studied substrates the hemicellulose content varies from 234 to 272 g/kg, and the acid detergent lignin content - from 26 g/kg to 57 g/kg. The lowest content of acid detergent lignin was detected in *Panicum miliaceum* substrates. The biochemical methane potential varied from 302 to 365 L/kg VS. The highest biomethane potential was achieved in *Panicum miliaceum* silage substrate. There are different results reported in research studies conducted by other authors. Ahn et al. (2010) studied the effect of co-fermentation of manure with switchgrass silage noted that switchgrass silage with swine manure had 373 L/kg methane, in mixtures of switchgrass silage and dairy manure 28 L/kg methane, while in mixtures of switchgrass silage and poultry manure 2 L/kg methane. Massé et al. (2011) reported that correlation of nitrogen rates and harvest dates and the methane yield of switchgrass silage substrate varied from 184 to 252 L/kg VS, while in reed canary grass silage

substrate, it varied from 5 to 253 L/kg VS. Frigon et al. (2012) found that, in the switchgrass harvested at the end of August, the concentrations carbohydrates were 2.4 g/kg arabinan, 18.5 g/kg xylan, 0.5 g/kg mannan, 1.34 g/kg galactan and 39.4 g/kg glucan, C/N ratio of 25:1, and the final methane production after 134 days of incubation in dependence of biomass pre-treatments varied from 157.0 to 297.7 L/kg VS. Niu et al. (2015) reported that in batch anaerobic digestion process of switchgrass biogas production was 268.80 L/kg VS and methane potential 135.31 L/kg VS, the conversion efficiencies of the material and energy from switchgrass to biogas were 36.1% and 30.1%. As a result of the analysis of the biogas produced from switchgrass by anaerobic digestion, Baute et al. (2018) found that the methane potential of *Panicum virgatum* varied from 160.1 to 186.5 L/kg VS, while *Miscanthus giganteus* substrate - from 167.5 to 229.8 L/kg VS, respectively. Barbanti et al. (2014) shows that switchgrass biomass had production methane potential of 217 L/kg, but maize substrate 316 L/kg VS. Battista et al. (2019) found the methane yield of *Panicum miliaceum* substrate was 253 L/kg, but *Zea mays* substrate - 289 L/kg, *Triticum aestivum* substrate - 351L/kg, *Hordeum distichon* substrate - 290 L/kg and *Sorghum* spp. substrate - 313 L/kg. Holder et al. (2022) found that the biochemical methane potential of the substrate from guinea grass, *Panicum maximum*, was 250 l/kg DM. Kupryś-Caruk et al. (2023) remarked that the methane yield of *Panicum virgatum* pure silages was 310 L/kg ODM, while the methane yields of inoculated silage reached 380 L/kg ODM.

CONCLUSIONS

The common millet, *Panicum miliaceum* and the introduced taxa of switchgrass *Panicum virgatum* are suitable for cultivated under the conditions of the Republic of Moldova and the harvested biomass may be used as fodders for livestock, also as substrates for biomethane production as a source of renewable energy.

ACKNOWLEDGEMENTS

This study has been supported by the subprogram no. 01.01.02 "*Identification of valuable forms of plant resources with multiple uses for the circular economy*".

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ISSN 2285 – 5785
ISSN-L 2285 – 5785