



UNIVERSITY OF AGRONOMIC SCIENCES  
AND VETERINARY MEDICINE OF BUCHAREST  
FACULTY OF AGRICULTURE



# SCIENTIFIC PAPERS

## SERIES A. AGRONOMY

VOLUME LXIV, No. 2



2021  
BUCHAREST

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# SOIL SCIENCES



## USE OF REMOTE SENSING TECHNIQUES AND GEOGRAPHIC INFORMATION SYSTEMS TO IDENTIFY DEGRADED LAND IN DHI QAR REGION FROM IRAK

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### Abstract

*This study was conducted to monitor the manifestations of land degradation in the area located within the administrative boundaries of the governorates of Dhi Qar, Qadisiyah and Muthanna, which has a surface of 37,984 km and specified by the space coordinates of Path = 167 and 38 = Row with the help of remote sensing technology through the use of satellite visuals from Landsat 8 satellite with field observations. A map of soil units was prepared using the software Arc GIS. Soil samples were taken from each site for physical and chemical analysis. The results obtained from the research showed that when using the spectral index represented by the plant difference index (NDVI) its value ranged between (-0.68 to 0.78). It was found that the study area suffers from a lack of vegetation cover by 72%. As for the salinity index values, they ranged between 0.68 to -0.78. Therefore, the study area suffers from a chemical deterioration represented by the salinity of the soil, estimated at 61%. For arid lands, the index ranged between -399.4 to -28.30, and the total surface of decertified lands represented 45%. As for the land degradation index, its value ranged from 1.21 to 2.84 and the percentage of degraded lands was estimated at 75% of the total lands that were study.*

**Key words:** degradation, remote sensing, geographic information systems, land degradation index.

### INTRODUCTION

Soil is one of the natural resources that plays an essential role in human life and therefore it is necessary to manage this resource efficiently to face the tremendous growth in population numbers.

The optimization of land resources is the main factor driving a significant increase in global food production.

For the purpose of exploiting the land resources, this process requires a use planning which is in turn a systematic assessment of the land and water potentials, alternative patterns of land use, and other physical, social and economic conditions.

Many researchers have endeavored to define the concept of soil degradation, the main cause of desertification (Al-Jawad, 1996).

The process of soil degradation is a result of wrong practices of agricultural lands that cause low productivity in the present and the future, which has a direct relationship to human life.

Degradation occurs all over the world, whether in developed or developing countries, the

percentage of agricultural land degradation in dry areas has reached 70% of the countries of the world (Farahat, 2000; Al-Azzhami, 2001).

Farahat added that more than 110 countries were affected by desertification such as the United States of America, but it was more severe in South Africa, then North and South America and European countries, especially Portugal, Spain, Greece and Italy.

Desertification has many concepts, Dograme (1999) defines desertification as the process of changing different ecosystems, the main cause of which is the misuse of natural resources (soil, plants, water) by humans.

The Arab Organization for Agricultural Development in 2001 clarified the main factors causing desertification and land degradation.

The organization pointed two main factors, namely the human factor, which is the misuse of natural resources, rapid population growth, overgrazing, logging and wrong plowing for agricultural purposes and the second factor is represented by natural factors such as the impact of climatic fluctuations and recurrent droughts which lead to degradation of

vegetation cover, soil degradation, sand dune movement, and land degradation and waterlogging. Therefore, the sustainability of these lands is a major goal of the sustainable agricultural development that aim to achieve self-sufficiency which requires the use of modern and successful management methods that help increase productivity while preserving lands from degradation processes (Ziboon et al., 2015).

## MATERIALS AND METHODS

The studied area is bordered from the north-west by Al-Muthanna Governorate, from the south-west by Dhi Qar Governorate, from the south-east by Al-Qadisiyah Governorate, and from the south-west by Basrah Governorate, with a surface of 37983.892 km and is located between two longitudes. Figure 1 shows the studied area and some of it's main features.

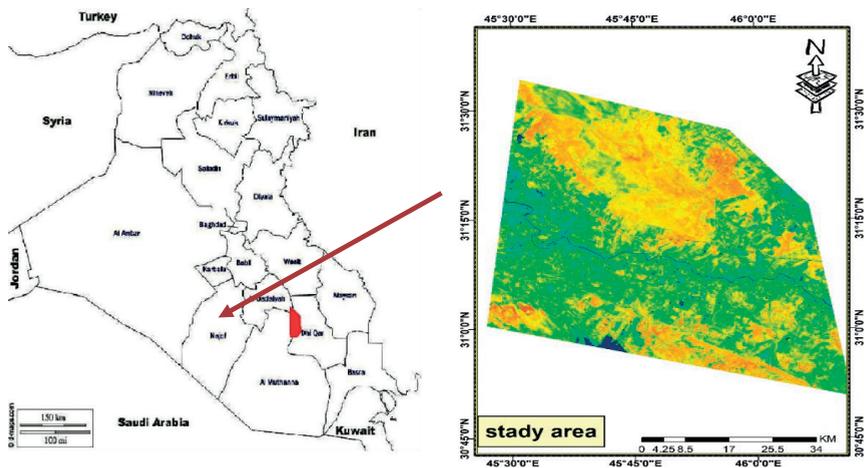


Figure 1. The Map of the study area

### Physical and chemical analyzes

The soil material samples taken from the horizons of the Bedoon soil were analyzed as followed: Quantify the size distribution of soil particles according to the absorbent method described by Day (1965). The bulk density was estimated by the Clod method according to Black (1965). Organic matter according to Walkely and Black method. EC electrical conductivity, Calcium sulfate, calcium carbonate according to the method mentioned in Handbook, USDA.

### Visuals and analyzing the satellite data

The satellite images provide a lot of information about spatial space, especially the multi-spectral visuals (MULTISPECTRAL IMAGERY), the information includes data about of the number of beams, obtained after the processing satellite visuals. One scene that covered the study area included the sensor data ETM obtained by the American satellite Landsat 8, while the other visual data for the sensor OLI for the year 2020 was obtained

from the USGS site as a punch in the plates (-) and the existing ones. Maps (1 & 2) were used to classify the land cover of the study area, to determine the most important aspects of degradation, to derive vegetation cover and sites of its spread and to determine its areas.

### Digital indexes

Digital index represents one of the most important improvements applied to satellite visuals, as it is the best choice for detecting differences that cannot be observed in visuals applied with basic color beams. The most important evidences are:

The Normalized Difference Vegetation Index (NDVI), as indicated by the formula defined by (Rouse et al., 1974).

$$NDVI = ((NIR-Red))/((NIR+Red))$$

$$NDVI = ((B5-B4))/((B5+B4))$$

As: NIR: Near infrared, B: Band

### Salinity Index (SI):

As in the formula indicated by (Khan et al., 2005).

$$Salinity\ Index = (B3 * B4) \setminus B2$$

### **Barren soil Index (BSI):**

As in the formula indicated by (Jamalabad and Abkar, 2004).

$$BSI = ((SWIR+Red)-(NIR-Blue))/((SWIR+Red)+(NIR+Blue)) + 1$$

$$((B6+B4)-(B5+B2))/((B6+B4) + (B5+B2)) + 1 = BSI$$

Whereas: NIR: Near infrared, B: Band

### **Land Degradation Index (LDI)**

This index was used in 2010 by Zhao and Meng according to the following formula:

$$LDI = ((255-(B2+B3))/(255+(B2+B3)))$$

Land degradation index can be determined by using Inverse Distance Way IDW for obtaining the soil specification maps in Information Systems Environment.

## **RESULTS AND DISCUSSIONS**

### **Physical attributes**

The laboratory results and field observations showed that there are important differences in soil properties between the studied sites and within relatively short distances, despite the fact that the parent material for all soils of these sites is developed from alluvial-Lacustrine in addition to some wind sediments (Al-Aqidi, 1986). There is a stratification of different textures. It turns out that the soils representing the two paths fall under the group Typic Torrifluvents which are characterized by the following. It does not have a horizon with a thickness of more than 15 cm during the first meter of the soil surface, containing more than 20% solid knots (durinodes) in a ground that is not fragile and has a solid cohesion when it is wet. It does not possess the following characteristics: Cracks for periods and for most of the years within 125 cm of the soil surface, which is 5 cm or more in width and 30 cm or more in depth. More than 30% clay from horizons whose total thickness is more than 50 cm within the first meter. The results of the analysis of the particle size distribution in Table 1 showed that there are differences in the textures of the horizons of the sites, as the boundaries between them are clearly distinguished, and the degree of clarity is proportional to the percentage of the soil content of clay and silt. The surface tissue was 0-30 cm for the eight headquarters loam at five sites and silt loam at site A2, while the tissue

for sites A4 and A8 and near the river was sandy loam. And, most likely, this difference in the percentage of time for the eight sites is due to the different intensity of the erosion processes represented by abrasion and the ablation that these sites were subjected to due to differences in the nature of land use and the quality and density of their vegetation cover, which in turn produced disparities in the depths of the different surface horizons where we find a continuous agricultural vegetation. The results of Kubota et al. (2005) showed that there was a significant decrease in the thickness of the surface layer with increasing soil roughness. He attributed these changes in the thickness of the horizon to the different effect of soil erosion with different tissues. In contrast, the depths of the subsurface horizons were not affected due to the limited erosion work at the surface. On the other hand, the textures of the subsurface horizons ranged from sandy loam to clay, this distinction in tissues may be due to the location of each mud from the supposed source of processing (the Euphrates River), which is consistent with West et al. (1980) results that confirmed the increase of clay in the soil content. The fine and medium silt increased in the logarithm of the distance from the source of the supply, while the proportion of coarse fractions decreased in the soils of the American floodplain. The average bulk density for all depths was 1.45  $\mu\text{g. M}^{-3}$ , as the highest value for this characteristic was recorded in the surface layer of the site A4 and the depth of 60-90 cm for the site A3, at 1.58 micrograms.  $\text{M}^{-3}$ , while it decreased to its lowest level at 30-60 cm depth at the A4 site. The soil bulk density rates of the sites can be assessed as falling within the natural levels that do not impede or prevent the growth of plant roots, according to SSI Report, 2004. The bulk density values reflect the nature of the size distribution of site soil particles. Kubota et al. (2005) stated that soils with a low content of clay and a high proportion of fine sand and silt tend to be compacted due to the expansion and contraction factors that accompany the conditions of wetting and drying.

The differences can also be attributed to the effect of the moisture content on these soils. This is confirmed by Alexander (1980)

regarding the correlation of the bulk density value with the water content of sedimentary soils where the largest value of bulk density appears with the water content below the permanent wilting point -15 bar. As the bulk density is highly dependent on soil conditions, changes in soil volume resulting from water content will change the bulk density values that are a function of different soil properties rather than a single value, according to Soil Survey Investigations Report, 2004. These differences can also be explained by the fact that the bulk density is related to the characteristics of the natural soil, which include tissue, organic matter and soil construction. The bulk density is variable throughout the year due to many

processes such as freezing, thawing, soil disintegration by drought, the effect of agriculture, animal activities, plant roots and tillage processes. The total porosity of the soil which expresses the spaces occupied by water and air, as well as the degree of soil compaction, influence the general rates of depths 0-30 (44.21%), 30-60 (46.02%), 60-90 (45.25%). Dorner (2010) confirmed that the porosity of the soil acts as a direct measure of the bulk density and explained the relationship between the moisture content to the porosity which explains the decrease of the porosity in soil that can be explain my the decrease of the surface area necessary for the movement of water in soil.

Table 1. The physical characteristics of the study soil materials

Point	Clay, %	Silt, %	Sand, %	Texture	Bulk density, g/cm <sup>3</sup>	Density, g/cm <sup>3</sup>
A1	24.15	34.88	40.97	Loamy	1.39	2.63
A2	8.12	51.45	40.43	Silty Loamy	1.44	2.68
A3	8.51	47.24	44.25	Loamy	1.36	2.59
A4	7.88	30.07	62.05	Sandy Loamy	1.58	2.6
A5	25.56	33.77	40.67	Loamy	1.35	2.61
A6	13.41	45.22	41.37	Loamy	1.49	2.6
A7	7.89	44.67	47.44	Loamy	1.39	2.63
A8	7.41	31.46	61.13	Sandy Loamy	1.49	2.65

### Chemical properties

The results showed that the general average of saturated aqueous conductivity values amounted to 0.246 m day<sup>-1</sup>, within a range between 0.433 m day<sup>-1</sup> at the 0-30 cm depth for site A4 and decreased to reach its lowest levels by 0.056 m day<sup>-1</sup> at depth 60-90 cm for site A1. The same method increased the general rate of this characteristic to the three depths for A4 location that recorded an average of 0.359 m day<sup>-1</sup>, while it decreased for the A1 site to 0.132 m day<sup>-1</sup>. These values indicate a high water loss rate for site A4 of 131 meters per year, compared to 48 meters per year for A1 site, which leads to an increase in the speed of water penetration into the depths of the soil and a decrease in the storage capacity of those sites. These differences in the Ksat values between the three sites may be mainly due to the soil texture, as sandy soils with large pores have a higher water conductivity than soft tissue soils that have small pores and higher porosity. As confirmed by Cornelis et al. (2001), the tests showed that the correlation relationship between the Hydrophilic properties and particle

sizes are very good for soils with high sand content, but they are not as accurate for soils with high clay. Zeleke et al. (2005) explained the reason for the great influence of mud on the values of water conductivity in comparison with the soil content of sand and silt due to the swelling and contraction characteristic, which determine the number and size of the flow paths. The saturated water conductivity of the sites' soils can be classified on the basis of their general rates into a fast class of highly desertified soils and a medium velocity classification for both medium and light desertified soils according to an assessment (USDA-NRCS, 2002). The results showed that the soil of site A6 recorded the highest pH for the three depths, reaching 8.48, while site A8 recorded the lowest value for this characteristic with an average of 7.81. Despite the differences in the chemical and physical properties of the soil of the eight sites, the presence of high levels of calcium carbonate led to the regulation of the values of soil interaction degree in the different sites ranging from mild to moderate alkaline according to the Soil

Survey Division Staff (1993). This indicates the high regulatory capacity of these soils due to

their high calcium carbonate content, as confirmed by Doner and Lynn, 1989.

Table 2. Chemical properties of the study soil materials

Point	Capillary rise, mm/day	Field capacity, %	Williting point, %	Ksat, mm/day	Total porosity, %	ECe, Ds m <sup>-1</sup>	pH	CACO <sub>3</sub> , G kg <sup>-1</sup>
A1	2.576	35.34	16.758	0.2475	47.148	78.8	7.72	243
A2	0.784	31.752	12.348	0.181	46.269	26.2	7.88	218
A3	2.208	31.62	14.994	0.285	47.490	12.15	8.12	275
A4	0.735	24.304	9.3	0.4332	39.231	9.51	7.34	229
A5	2.806	37.82	17.934	0.255	48.276	79.6	7.56	208
A6	2.392	32.86	15.582	0.265	42.692	34.1	8.16	217
A7	2.139	30.69	14.553	0.305	47.148	12.65	7.17	206
A8	0.651	20.181	11.2	0.3876	43.774	10.14	7.44	233

### Normalized Difference Vegetation Index (NDVI)

The results indicated that the NDVI values are situated between 0.25 to -0.25. The reason is that there is a clear variation in the distribution

of the type of land cover prevailing in the studied area and in a disparate manner distributed between agricultural lands, natural plants and plants growing in the areas near the river (Shallal et al., 2007).

Table 3. Ranges of land degradation and its relationship to the NDVI

NDVI Range	Area, km	Veg. Cover ratio	Land Deg.
Non Veg	29114.45	77%	Hight Deg.
Moderate veg	6850.026	18%	Moderate Deg.
Very veg	1703.619	4%	Non Deg.

The absence of vegetation cover an occupied area estimated at 29114.45 km from the total area of the study, with a 77% due to the presence of arid lands affected by salinity and desertification. It covers an area of about 6850.026 km (14%) due to the high rate of salinity and the impact of difficult climatic conditions, the lack of rain and high temperatures that affected the growth of plants,

which led to the reduction of vegetation cover of the area. As a result, most of the lands that comprise the studied area are not for agricultural use. As the vegetation covered area, it is estimated at 1703.619 km (4%), and the reason is due to the fact that these lands are located in the river banks or it is an agricultural use of crops or economic farms (Table 3, Figure 2) (Ibrahim, 2008).

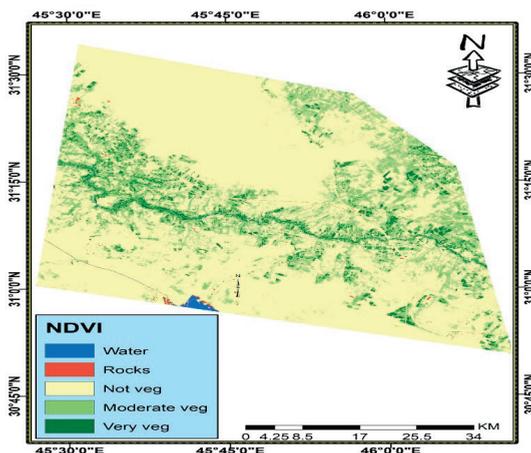


Figure 2. A map showing the Natural Vegetation Index (NDVI)

### Salinity Index (NDSI)

The results showed that the salinity index values ranged from -0.45 to -0.30. The results shown

in Table 4 and Figure 3 indicate that there are 5 categories that represent the salinity index values in the study area (NDSI).

Table 4. Ranges of the salinity index values

NDSI range	Area of salinity, ha	Salinity cover ratio
Not salinity	1561.86	4%
Low salinity	4840.614	13%
High salinity	31239.94	82%
Very high salinity	203.121	1%
Water	138.366	0%

The salinity category occupied an area estimated at about 1561.86 km of the total area (4%) as it is cultivated land or located on the rivers banks and this is considered natural drainage. The low salinity category occupied an area of 4840.614 km (13%). The salinity reflects the poor quality and scarcity of water, not the low percentage of cultivated land. The salinity is due to the fluctuation of groundwater levels and the high salt content in them. It has

effectively contributed to the emergence of soil salinization processes, which negatively affected the growth and development of vegetation cover and the deterioration of the physical and chemical properties of the soil, while the highly saline and saline variety occupied an area of 31239.94 (82%) and 203.121 km (1%), respectively. This indicates the presence of desertified soil (Al-Zubaidi et al., 2003).

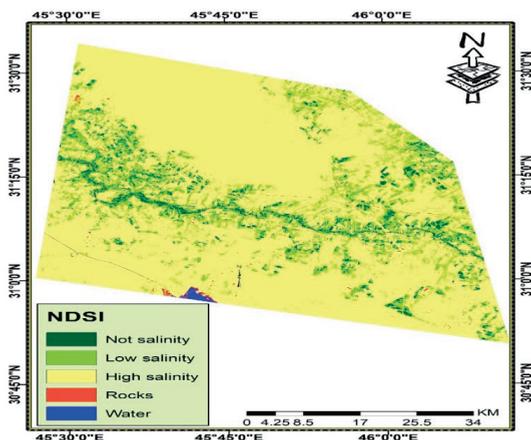


Figure 3. Map that shows the salinity index

### Barren Soils Index BSI

The values of the arid land index ranged between (0.25-0.1), possibly due to the high level of salinity and the impact of difficult climatic conditions, including the lack of rain

and high temperatures, which affected the growth of plants, which led to a decrease in the area of vegetation cover and land degradation (Mahdi, 2019).

Table 5. Ranges of degradation to the study land according to the Barren Soils Index (BSI)

Range BSI	Area of class	Ratio	Land degradation
<zero	4187.772	11%	Non-deg.
0.0-0.1	9067.374	24%	Light-Deg.
0.1-0.25	8050.023	21%	Moderate Deg.
0.25-0.5	9267.291	24%	High Deg.
>0.5	7411.437	20%	Very high Deg.

The non-desertified type occupied an area estimated at about 4187.772 km (11%) of the total studied area which is located in the rivers slopes and is exploited in agriculture, while the land with little desertification occupied large surfaces within the study area 9067.374 km representing 24%. As for the medium type in desertification, it occupied 21% of the area. This may be due to the fluctuation of

vegetation cover in these lands, the low percentage of cultivated lands and the existence of dry water bodies (dry swamps). Desertification severe occupies an area of 9267.291 (24%) and moderate desertification total sum of 7411.437 km (20%), which is determined by the high percentage of salts and the absence of vegetation cover (Table 5, figure 4).

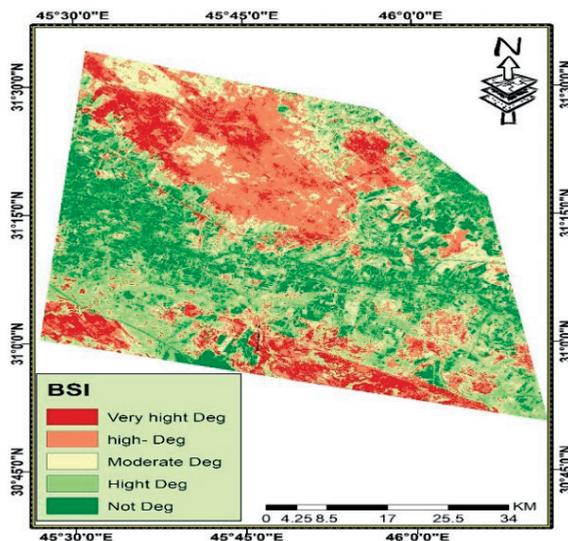


Figure 4. Map that shows the barren soils

### Land Degradation Index (LDI)

The results showed that the values of the Land Degradation Index ranged in Figure 5 between 1.2-2.8. This is due to the fact that most of the lands of the study were affected by the high temperatures and the increase in the percentage

of salts in the accompanying soil. The transfer of groundwater with capillary property to the surface of the soil and its evaporation, caused the accumulation of salts that formed a layer of salt marsh soil, being considered one of the main causes of its deterioration (Al-Rawi and Al-Jorsi, 2014).

Table 6. Shows the ranges of lands degradation according to LDI at the study area

Type of Deg.	Ratio	Area KM
Water	13%	5102
Non-degraded	24%	9301.54
Moderate degradation	21%	8262
Severity degraded	30%	11607
Very sever degradation	12%	4712

The non-degraded variety reached an area estimated at 5102 km (13%) of the total area and the reason for this is the presence of these sites in rivers. As for the lands of light degradation, they measured a surface of 9301.54 km (24%), which is occupied by

agricultural land with crops and economic farms. As for the medium deterioration, it summed 8262 km (22%), the main reason being the influence of the semi-arid climate, the lack of rainfall and the mismanagement of irrigation, causing the degradation of these

lands, in addition to the conditions prevailing in the region (Muhammad, 2017). The type of severe deterioration and very severe deterioration reached the area estimated at about 11607 (30%) and 4712 km (12%) (Table 6). The reason for the existence of deterioration may be attributed to the vast areas devoid of

vegetation as a result of the influence of the prevailing climate. Conditions in the study area varies from high temperatures, lack of rainfall, high salinity in the soil, as well as poor management irrigation and drainage operations that affect the growth of plants.

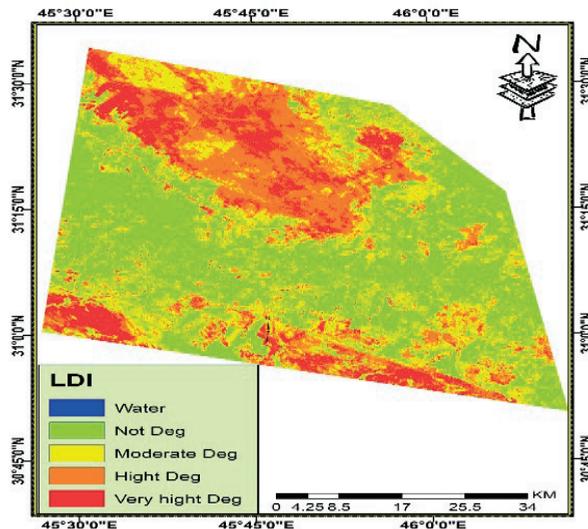


Figure 5. Map of land Degradation index (LDI)

## CONCLUSIONS

The study demonstrated the ability of remote sensing and geographic information systems to survey, monitor, identify, analyze and classify aspects of land degradation in the study area and detect spatial and temporal changes that may occur.

Landsat satellite visuals are used for regional studies that are conducted over a relatively large area due to the relatively low spatial resolution, which ranges between 6-15 meters, the accuracy of its results increases through spectral, spatial improvements and matching operations with reference information, whether it is maps or pictures.

The digital indicators (INDEX) contributed to highlighting the land features very effectively in clarifying the spatial distribution picture and the quantitative and qualitative assessment of the types of land degradation manifestations in the study area, such as the visuals resulting from the natural difference index of vegetation

cover, Salinity Guide, Arid Soil, Soil Degradation Guide.

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## GENETIC PECULIARITIES OF THE NATURAL AND AGRICULTURAL GREY SOILS FROM THE REPUBLIC OF MOLDOVA FOREST-STEPPE

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### Abstract

*Some genetic peculiarities of natural and arable Grey Forest Soils (Greyzem) are presented in order to improve their classification. The textural differentiation of the typical (natural) grey forest soil profile from the northwest of the Northern Moldavian Plateau occurred under the action of three pedogenesis processes: in situ alteration of the soil material; leaching of the alteration colloidal material; migration on the profile of water-soluble organic-mineral compounds of Fe and Al in the podzolic process. The weakly textural differentiation of the arable grey soils profile is due to the partial inclusion of the illuvial horizon in the arable layer with a thickness of about 35 cm, the intensification of the in situ alteration process in the arable layer of these soils due to the change of reaction and hydrothermal regime as result of agriculture use. The textural differentiation of the grey forest soils profile in complex with other indicators is an important diagnostic index for improve the classification status of grey forest soils from forest-steppe of the Republic of Moldova.*

**Key words:** Grey Forest Soils, classification, texture differentiation, colloidal material, in situ alteration.

### INTRODUCTION

The development of soil science (pedology) at international and national level, the progress of soil knowledge in recent times, determines the need to re-examine and clarify the principles and criteria of soil classification (both higher and lower level), focusing on their concrete properties and appreciation of the factors and processes of pedogenesis that led to the formation or modification of some features.

At present, national soil classifications bear considerable influence from the soil classification system in the Legend of the World Soil Map (FAO-UNESCO, 1990) and the World Reference Base for Soil Resources (WRB, 2006, 2014). The radical change of the concept of classification, of the nomenclature and the scientific notions regarding the soils, sometimes makes impossible the use of the materials of the previous pedological researches. For these reasons, the transition to a completely new classification of Moldovan soils is not rational. Therefore, in order to improve the soil classification system, the collection, systematization, analysis and evaluation of archive and current materials were used. At the same time, field research was

carried out by placing soil profiles and collecting soil samples in order to materialize some genetic features.

In the elaborating the improved grey soils classification, it is necessary that for some soil taxonomic units of the national classification, to find adequate names for the soil nomenclature of Moldova from the Romanian soil taxonomy system (Vlad et al., 2014), as well as the use of standards and diagnostic elements of these classification systems. Although elements and standards from other soil taxonomy systems will be introduced in the improved soil classification system, the main traditional elements of the pedology of the Republic of Moldova, based on the Russian naturalistic soil classification system (Иванов, 1956; Крупеников, 1973; Егоров, 1977; Ursu, 2001, 2006; Cerbari, 2001).

The partial agreement of the soil classification system in the Republic of Moldova with the classification system in the Legend of the "Soil Map of the World" (1990) and the "World Reference Base for Soil Resources" (2006, 2014) will contribute to the dissemination of information on soil variety of the Republic of Moldova internationally; partial unification of the soil taxonomic units system and diagnostic

characters used; correlation of the soil map of the Republic of Moldova with the soil map of Romania and Russia, other countries, as well as with the Soil map of the World

The particularities of the genesis, classification and geographical spread of the Grey soils, formed under the forest vegetation are reflected in a series of researches (Крупеников & Скрябина, 1976; Урсу & Крупеников, 1984, Ursu, 2011; Cerbari et al., 2011, 2017; Cerbari, 2010; Leah, 2020). According to the mentioned authors, the genesis of forest soils of Moldova has common characteristics with forest soils in the Eurasian Steppe, Central and Eastern Europe, Romania and Bulgaria.

Thus, it was established that in Central Moldova the most widespread are the typical Grey soils. The illuvial horizons of these soils are formed as a result of the combination of the podzolic process and the alteration material of this "in situ" horizon (Reabinina, 1968; Хотинский, 1986). Then, the classification of the soils from "Codri" was improved at the level of genus, species and variety of soil, the agronomic grouping of the soils was performed, the suitability of Grey soils for different use was assessed. Of particular interest is the description of the stagno-gleic phenomena located in the lower part of the profiles of some grey forest soils (Balteanschi, 1979).

The research on the genetic peculiarities continued with the observations made in stationary with lysimeters on the Grey soils of the forest and on the arable lands (Ivancea commune, Orhei district). These researches coincided with a comparatively humid climatic period 1960-1964 that was established on the territory of Moldova, which led to the intensification of the processes of argillisation, leaching and lessivage (Адаменко, 1996; Grati, 1975, 1977).

Thus, the Grey forest soils of Central Moldova differ from most forest soils in the forest-steppe area of Northern Moldova, Ukraine and Russia by the lack or very weak development of the podzolic process and its replacement with the process of comparatively low intensity lessivage and the process of "in situ" alteration of the underlying horizons (Ахтырцев & Щетинина, 1969; Полупан, 1986; Лунгу, 2008; Лях, 2017; Cerbari et al., 2017).

Currently, for practical purposes is used "Soil Classification of the Republic of Moldova",

developed by Ursu (2001). In this classification the Grey soils are divided into four subtypes: albic, typical, mollic and vertic.

The research aim was to evaluate the changes in the properties of Grey soils as a result of arable use and changes in the pedogenesis phases; improving the classification of Grey soils and highlighting soil taxonomic units.

## MATERIALS AND METHODS

Classical research methods (Аринушкина, 1970; Агрохимические..., 1975) to perform the studies and criteria for evaluating soil properties (Florea et al., 1987; Jigău et al., 2007) were used. The Grey soils (virgin and arable) from the north-western part of the Northern Moldavian Plateau served as research objects. In order to fulfil the proposed aims, the soil profiles were placed in the virgin forest and on the arable land.

**Grey soil typical** (natural or virgin), located in the oak forest in the western part of Briceni city, on a horizontal surface of the plateau. Absolute altitude - 258 m. Latitude - 48.35466. Longitude - 26.99946. The parental rocks are presented by strongly altered loess deposits. The surface coverage with grasses and shrubs is 20-30%. The Grey forest soil is characterized by the type of profile: AEh 0-8 cm → AEh 8-29 cm → EBhtw 29-47 cm → Btw 47-68 cm → BCtw1 68-96 cm → BCtw2 96-110 cm → Ck 110-130 cm (Figure 1).



Figure 1. Profile of Grey soil (Greyzem) typical, clayey-loamy, forest

**Grey soil cernic**, arable, located on the horizontal surface of the “Movila Mare” height, opposite of Larga village, Briceni district. Absolute altitude - 248 m. Latitude - 48.38200. Longitude - 26.85202. Arable Grey soil is characterized by a profile of the type: Ahp1 0-12 cm →Ahp2 12-30 cm →Bh1 30-45 cm →Bh2 45-65 cm →BC1 65-90 cm →BC2 90-110 cm →Ck > 110 cm (Figure 2).

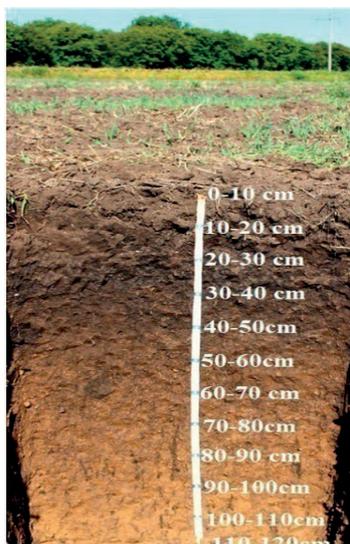


Figure 2. Profile of Grey soil (Grezem) cernic, clayey-loamy, arable

The profile of arable Grey soil, compared to the profile of natural grey soil, is not differentiated textural (Figures 1 and 2). This is the result of the periodic restoration on this soils of the secondary steppe vegetation and its use in arable land during the Cucuteni-Tripole culture, 2000-4000 years until our era (Florea, 2005; Xenopol, 2006). This soil has a polygenetic origin, that is, it was formed as a result of deforestation of the virgin forest long ago and use of land for agriculture. According to historical sources (Докучаев, 1950; Гроссер, 1961; Александровский, 2006) during the migration of peoples from East to West, the land used for agricultural purposes was abandoned. Thus, on these soils, the natural vegetation was restored and the pedogenesis process was modified, towards the chernozems formation. As result of local climate Greyzems were formed.

## RESULTS AND DISCUSSIONS

The characteristic of Grey soil properties was made based on the results obtained for the Grey soil profiles (forest and arable) in the north-western part of the Northern Moldavian Plateau. This territory is characterized by absolute heights within the limits of 242-284 m and a wetter climate than in the other administrative districts located on this plateau.

Texture is the main physical property of the soil with a particularly important role in determining most of the other physical properties, as well as many chemical properties (Canarache, 1990). In wetter areas (e.g. forests), where during the soil genesis there are intensification processes of argillisation and clay migration, soil profiles are formed with deep textural differentiations (Лунгу, 2007, 2008). In general, profiles with textural differentiation are presented by the soils with horizons of clay formation and accumulation (Btw, BCtw etc.).

**Grey soil typical** in the north-west is characterized by clearly differentiated texture, by the eluvial-illuvial process and pedogenesis on the depth of the profile (Figure 1, Table 1).

For quantitative expression of the textural differentiation between horizons, the *textural differentiation index (Idt)* is used which represents the value of the ratio between the percentage of clay in horizon B and the percentage of clay in horizon A. The index of textural differentiation, compared to the eluvial horizon, reaches values 1.7-1.9. Soils with such *Idt* values are appreciated as moderately differentiated texture of soils (Figure 3).

The textural differentiation of the typical grey soil profile occurred under the common action of the following processes (as mentioned above in the text): “*in situ*” alteration of the soil material of the eluvial horizon; leaching the alteration colloidal material; podzolic process - migration of soluble organo-mineral Fe and Al compounds in the profile (Зонн, 1976).

However, in the opinion of the authors, in this concrete case the main role in the textural differentiation of the researched typical Grey soil profile belongs to the podzolic process. Indirectly, this is confirmed by the value of  $pH_{KCl} = 4.1-4.3$  of the eluvial - illuvial horizons in the depth of 27-96 cm (strong and moderate

acidity). At the same time, the values of hydrolytic acidity at 27-96 cm are medium and vary within the limits of 4.1-5.9 me (Table 2). In the researched profile, the soil color of the eluvial horizons is grey, and of the illuvial horizons - dark brown (Figure 2). According to the data the thickness of the humiferous profile is 47 cm. According to the

humus content, the genetic horizons of the researched soil can be assessed as follows: AEht (0-8 cm) - high content; EAh (8-27 cm) - submoderate content; EBhtw (27-47 cm) - small content. The other underlying horizons are characterized by a humus content of less than 1.00% (Figure 4).

Table 1. Texture of Greyzems from the north-western part of the Northern Moldavian Plateau

Horizon and depth, cm	Dimensions of fractions (mm); content (%)						
	1-0.25	0.25-0.05	0.05-0.01	0.01-0.005	0.005-0.001	<0.001	<0.01
<i>Grey soil (Greyzem) typical, submoderate humiferous with semi-deep humiferous profile, clayey-loamy 0-47 cm, loamy-clayey &gt;47 cm</i>							
AEht 0-8	0.4	14.1	39.1	8.9	16.9	20.6	46.4
EAh 8-27	0.2	11.2	40.5	9.8	16.8	21.5	48.1
EBhtw 27-47	0.2	8.4	40.7	6.3	15.7	28.7	50.7
Btw 47-68	0.1	6.4	30.5	8.2	18.5	36.3	63.0
BCtw 68-96	0.1	8.4	29.9	9.7	14.2	37.7	61.6
BCtw 96-110	0.1	9.7	28.6	9.5	12.4	39.7	61.6
Cwk 110-130	0.3	9.1	28.2	9.7	15.1	37.6	6.4
<i>Grey soil (Greyzem) cernic, submoderate humiferous with semi-deep humiferous profile, clayey-loamy</i>							
Ahp1 0-12	0	19.2	26.0	5.2	15.7	33.9	54.8
Ahp2 12-30	0	20.8	24.7	5.3	15.6	33.6	54.5
Bh1 30-45	0	14.4	30.8	9.1	14.9	35.4	54.8
Bh2 45-65	0	11.4	31.0	8.1	13.3	36.2	57.6
BC1 65-90	0	10.8	31.6	7.8	13.2	36.6	57.6
BC2 90-110	0	10.5	32.4	7.6	12.1	37.4	57.1
Ck 110-130	0	11.3	30.9	7.6	12.9	37.3	57.8

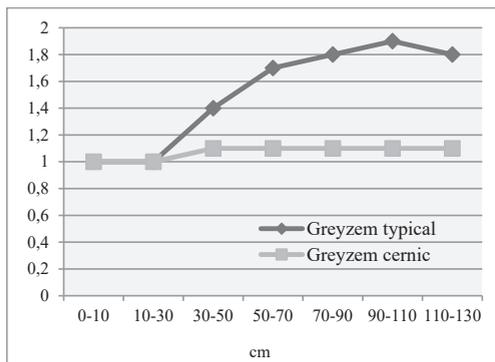


Figure 3. Index of textural differentiation (Idt) values of the Greyzems

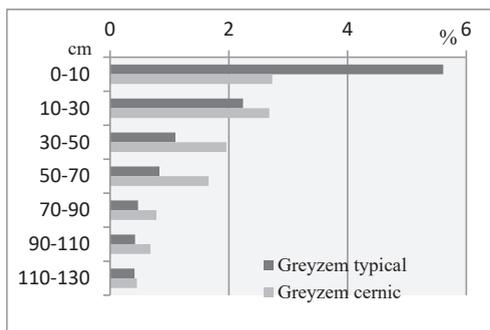


Figure 4. Humus content in the Greyzems

The carbonates content on the profile of the researched soil are leached deeper than 110 cm. The sum of exchangeable cations is medium in the depth 0-68 cm and large in depth > 68 cm. The value of the ratio Ca:Mg is equal to 3.4-3.7 for the eluvial horizons; 5.1 - for the Btw illuvial horizon; 2.5 - for the illuvial horizon. In the Ctw and Ck horizons the exchangeable Mg<sup>2+</sup> content is higher than the exchangeable Ca<sup>2+</sup> content. A possible magnesium slitization of this horizon is not excluded (Table 2).

The hygroscopicity coefficient and the plant wilting coefficient for the typical Grey soil are characterized by moderate values. According to its properties and natural conditions, the natural grey soil can be appreciated as typical.

**Grey soil cernic** on the arable is characterized by homogeneous clayey-loamy texture on the depth (Table 1). This texture is favorable for growing field crops. Soils with medium-fine texture have a high fertility, if their arable layer is structured and loose. The tillage of the clayey-loamy soils is necessary to be carried out at the humidity corresponding to its friable state - the state of humidity at which the soil crumbles easily (Cerbari et al., 2017). The arable clayey-loamy Grey soils are comparatively resistant to drought as a result of

the high capacity to accumulate water in the cold period of the year. However, the water regime of compacted arable clayey-loamy soils is partially unfavorable. Therefore, the preventive restoration of the physical quality

state of destructured and compacted arable layer is absolutely necessary to be carried out until the implementation of conservative agriculture system (Cerbari & Leah, 2010).

Table 2. Physical and chemical properties of grey soils (greyzems) of the North Moldovan plateau

Horizon and depth, cm	*HW, %	*HC, %	Density, g/cm <sup>3</sup>	CaCO <sub>3</sub> , %	*pH <sub>H2O</sub>	*pH <sub>Kcl</sub>	me/100 g soil			
							*HA,	Ca <sup>2+</sup>	Mg <sup>2+</sup>	ΣCa+Mg
<i>Grey soil (Greyzem) typical, submoderate humiferous with semi-deep humiferous profile, clayey-loamy 0-47 cm, loamy-clayey &gt;47 cm</i>										
AEht 0-8	2.9	8.5	2.54	0	5.9	5.6	5.7	17.6	5.2	22.8
EAh 8-27	2.4	8.2	2.63	0	5.6	5.0	5.9	12.4	4.4	16.8
EBhtw 27-47	2.5	7.4	2.65	0	5.6	4.3	5.0	13.2	3.6	16.8
Btw 47-68	3.6	9.4	2.68	0	5.8	4.1	4.8	16.4	3.2	21.6
BCtw 68-96	4.0	9.7	2.68	0	5.9	4.3	4.1	18.0	7.2	25.6
Bctw 96-110	4.0	10.7	2.70	0	7.0	6.0	1.1	12.8	15.2	28.0
Cwk 110-130	3.8	10.5	2.69	5.9	7.0	-	-	12.8	17.2	28.0
<i>Grey soil (Greyzem) cernic, submoderate humiferous with semi-deep humiferous profile, clayey-loamy</i>										
Ahp 1 0-12	3.1	8.6	2.56	0	6.5	5.8	5.5	20.4	3.2	23.6
Ahp2 12-30	3.3	8.7	2.63	0	6.4	5.6	4.8	20.2	3.0	23.2
Bh1 30-45	3.3	9.4	2.62	0	6.4	5.6	3.2	18.8	3.6	21.6
Bh2 45-65	3.5	9.9	2.64	0	6.2	5.6	3.1	18.0	4.8	22.8
BC1 65-90	3.7	10.2	2.65	0	6.5	5.5	1.9	18.4	4.4	22.8
BC2 90-110	3.6	9.9	2.67	0	6.7	5.7	1.4	18.6	4.4	24.0
Ck 110-130	3.0	8.9	2.68	12.0	7.8	-	-	18.8	4.0	22.0

According to the data (Table 2), the arable Grey soil is submoderately humiferous with semi-deep humiferous profile and requires application of organic fertilizers. The pH value of the arable Grey soil is characterized by a weakly acid reaction, homogeneous on the profile. The pH values vary in the range of 6.5-6.7. The values of hydrolytic acidity vary on the arable soil profile in the limits of 5.5-5.8 me and can be appreciated as moderate. The sum of exchangeable cations varies in soil depth within 22-24 me. The Ca:Mg ratio is favorable. The researched arable soil is an automorphic soil provided with moisture and under normal fertilization conditions will permanently ensure high yields of agricultural crops.

The anthropogenic transformation of the soils represents a major change as a consequence of the human intervention on the environmental conditions through the land improvement works and implicitly on the pedogenesis process, of some mechanical interventions that strongly change the soil profile, of the improper use of the soils leading to degradation processes.

## CONCLUSIONS

Grey Forest soils (Greyzems) typical are predominantly widespread in the forests of the Northern Moldovan Plateau and were formed in conditions of moderately warm semi-humid

to humid temperate climate and low-contrast soil hydrothermal regime. The pronounced textural differentiation of the typical Greyzem profile of the Northern Moldavian Plateau occurred under the action of pedogenesis processes: "in situ" alteration; leaching of the colloidal material of the alteration; podzolic process of soluble organo-mineral Fe and Al compounds migration. They are characterized by a semi-deep humiferous profile and are submoderate humiferous.

The weakly textural differentiation of the arable Grey soils profile is due to: the partial inclusion of the illuvial horizon in the arable layer with a thickness of about 35 cm; intensification of the alteration "in situ" process in the arable layer as a result of the changes of the pH reaction and the hydrothermal regime of the soil as consequence of its use in agriculture. The tillage followed by the destructuring of the arable layer led to the loss of the compaction resistance capacity; decrease the humus content by 1.6-1.7 times; the apparent density at the depth of 10-30 cm (below the periodically cultivated layer) towards the middle of summer reaches values equal to 1.50-1.65 g/cm<sup>3</sup> (very high), and the degree of compaction - exceeds 20% (very compacted soil); as a result, the physical quality of this layer became unfavorable for the plants growth as well as the underlying illuvial layer.

Textural differentiation of the Grey soils profile (greyzems) in complex with other indicators is an important diagnostic index of these soils for the purpose of their classification. Thus, in the perfected Soil Classification of the Republic of Moldova, the natural Grey soil (forest) were called Greyzem typical, the arable Grey soils - Greyzem cernic.

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## CHANGE IN AGROCHEMICAL INDICATORS OF LEACHED CHERNOZEM IN NO-TILL TECHNOLOGIES

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### Abstract

*The article presents the results of studies on the study the No-till technology influence depending on mineral fertilizers on the change in the agrochemical parameters of leached chernozem. Studies have shown that the seven-year use of No-till technology without fertilizers led to a decrease in the content of alkaline hydrolyzable nitrogen in leached chernozem by 10.1-13.7 mg/kg of soil, mobile phosphorus by 10.3-16.0 mg/kg, mobile potassium by 9.5-13.1 mg/kg of soil. The application of mineral fertilizers in the form of ammonium nitrate, sulfoammophos and ammofoska increased the content of alkaline hydrolysable nitrogen in the 0-30 cm layer by 12.1-13.6 mg/kg. The content of mobile phosphorus increased only in the 0-30 cm layer in the variants with the use of phosphorus-containing fertilizers by 17.7-19.9 mg/kg, and the content of mobile potassium in the variant with the potassium-containing fertilizer by 3.7-12.3 mg/kg of soil. The use of complex fertilizers such as sulfoammophos and ammofoska did not lead to a sharp decrease in pH. An increase in acidity was observed only in the variant with the use of ammonium nitrate.*

**Key words:** No-till, mineral fertilizers, alkaline hydrolyzable nitrogen, mobile phosphorus, mobile potassium, pH.

### INTRODUCTION

Over the past 50-60 years, in countries developing intensive technologies for the cultivation of agricultural crops, the soil fertility of agricultural land has deteriorated more than twice. Traditional methods of intensive tillage sooner or later lead to a decrease in the stock of soil humus, a decrease in soil biological activity and (or) erosion up to soil degradation, as well as a decrease in productivity (Chekaev et al., 2015; Derpsch et al., 2010).

According to a number of authors, resource-saving technologies help preserve the level of humus content and reserves, mainly by reducing the loss of organic matter by slowing down the mineralization processes and increasing its labile part from the remaining plant residues (straw, chaff) (Huang et al., 1999; Vogeler et al., 2009; Martínez et al., 2016).

At present, the No-till technology is recognized as the highest level of minimization of soil tillage and sowing in the world. No-till farming can no longer be seen as a temporary fad or fad.

This system has established itself as an agricultural practice and a distinct way of thinking about the sustainable management of an agroecosystem. With the No-till system, the influence of nature and climate is reduced to 20%. The remaining 80% is accounted for by technology and management in agriculture (Derpsch et al., 2010).

In increasing yields with direct sowing, the decisive role is played by mineral fertilizers, of which nitrogen fertilizers are of paramount importance (Liang et al., 2016). The use of No-till technology preserves soil nutrients and promotes better absorption of nitrogen, phosphorus and potassium by plants, which leads to an increase in the economic efficiency of fertilizer use. (Yang et al., 2020; Borges et al., 2000; Medvedeva et al., 2020).

No-till technology assumes that no fertilizer is applied during the period from the end of harvesting of the precrop to sowing. The key to obtaining a high yield using this technology is the application of fertilizers during sowing and feeding during the growing season of the crop (Chekaev N. et al., 2015).

## MATERIALS AND METHODS

Studies to assess changes in agrochemical parameters of leached chernozem in No-till technologies with the use of mineral fertilizers were carried out in 2013-2019. at the experimental sites in LLC Kameshkirsky feed mill, Kameshkirsky district, Penza region, according to the following scheme:

1. Without fertilizers (control);
2. N<sub>20</sub> in the form of ammonium nitrate (60 kg/ha);
3. N<sub>20</sub>P<sub>20</sub>S<sub>14</sub> in the form of sulfoammophos (100 kg/ha);
- four. N<sub>20</sub>P<sub>25</sub>K<sub>25</sub>S<sub>23</sub> in the form of ammophoska (166 kg/ha);

The research was carried out in a crop rotation with alternating crops: peas (*Pisum sativum* L.) - winter wheat (*Triticum aestivum* L.) - sunflower (*Helianthus annuus* L.) - spring wheat (*Triticum aestivum* L.) - buckwheat (*Fagopyrum esculentum*). The soil of the experimental plots is represented by leached chernozem, low-humus, medium-thick, light clayey. The upper thirty-centimeter soil layer before sowing peas in 2013 was characterized by the following indicators: humus content 5.2 ... 5.6% (average content), pH (KCL) 4.9 ... 5.2 (weakly acidic), alkaline hydrolysable nitrogen 95.3 ... 106.7 mg/kg (low content), mobile phosphorus 67.3 ... 70.3 mg/kg (average content), mobile potassium 120.0 ... 136.0 mg/kg soil (increased content). The area of the accounting plot is 42 m<sup>2</sup>.

Fertilizers were applied during sowing with seeding machines for No-till technology. Laboratory analyzes of soil samples were carried out by the following methods:

- mobile compounds of phosphorus and potassium - according to the method of F.V. Chirikov (GOST 26204-91);
  - alkaline hydrolysable nitrogen - according to the Kornfield method (modified by CINA0);
  - pH of the salt extract on a pH meter (GOST 26483-85);
- Yield accounting - by the gravimetric method according to the variants of the experiment in 3-fold repetition from 1 m<sup>2</sup>.

## RESULTS AND DISCUSSIONS

Among the agrochemical parameters of the agroecological state of soils, traditionally, the greatest attention is paid to the content, reserves and quality of humus, available forms of basic nutrients (NPK) with assessment scales, pH values adapted to the conditions of a particular region.

Long-term application of no-till technology leads to differentiation of nutrient content in the upper and lower layers of the soil profile (Martínez et al., 2016; Messiga et al., 2012).

Mobile alkaline hydrolysable nitrogen characterizes the content of nitrogen potentially available for plants.

The content of alkaline hydrolyzable nitrogen in the 0-30 cm layer in the first year of the introduction of the No-till technology was in the range of 95.3-106.7 mg/kg soil, which was characterized by both very low and low content.

In the soil layer of 30-50 cm, the content of alkaline-hydrolyzed nitrogen was lower than in the upper 30-centimeter soil layer, and amounted to 84.5-99.2 mg/kg of soil (Table 1).

Table 1. Change in the content of alkaline hydrolyzable nitrogen in leached chernozem in No-till technology

Option	Soil layer, cm	Alkaline hydrolysable nitrogen, mg/kg soil		
		2013 (first year of implementation)	December 2019	Deviations from the original data
1. Without fertilizers (control)	0-30	106.7	93.0	-13.7
	30-50	99.2	89.1	-10.1
2. N <sub>20</sub>	0-30	95.3	107.4	12.1
	30-50	86.0	80.3	-5.7
3. N <sub>20</sub> P <sub>20</sub> S <sub>13</sub>	0-30	98.0	111.8	13.8
	30-50	84.5	77.0	-7.5
4. N <sub>20</sub> P <sub>25</sub> K <sub>25</sub> S <sub>23</sub>	0-30	99.6	113.2	13.6
	30-50	97.6	90.7	-6.9
NSR05	0-30			3.2
NSR05	30-50			2.7

Seven years after the introduction of the No-till technology (2019), the content of alkaline hydrolysable nitrogen was 93.0-113.2 mg/kg in the 0-30 cm layer and 77.0-90.7 mg/kg in the 30-50 layer cm. On the option without fertilizers, the content of alkaline hydrolyzable nitrogen decreased by 13.7 mg/kg in the 0-30 cm layer and by 10.1 mg/kg in the 30-50 cm layer. On the options with the introduction of mineral fertilizers, the nitrogen content in the 0-30 cm increased by 12.1-13.8 mg/kg. A decrease was observed in the 30-50 cm layer in all variants.

Thus, the use of nitrogen-containing fertilizers leads to the accumulation of alkaline hydrolyzable nitrogen in the upper 30-cm soil layer and to a decrease in its content in the 30-50 cm layer. At the same time, the dynamics of

a clear differentiation of its content in the soil profile begins to appear.

Phosphate regime of soils means their ability to supply plants with phosphorus, which is characterized by: 1 - capacity factor, meaning the amount of phosphorus available to plants; 2 - an intensity factor characterizing the degree of mobility of the stock of soluble phosphates available in the soil (or the possibility of using mobile phosphates by plants).

The content of mobile phosphorus in the first year of the introduction of the No-till technology was 67.3-70.3 mg/kg of soil, which was characterized as an average content for grain crops and low for fodder crops, root crops and potatoes. In the layer of 30-50 cm, the phosphorus content was lower compared to the 0-30 cm layer by 4.3-17.7 mg/kg (Table 2).

Table 2. Change in the content of mobile phosphorus in leached chernozem in No-till technology

Option	Soil layer, cm	Mobile phosphorus, mg/kg soil		
		2013 (first year of implementation)	December 2019	Deviations from the original data
1. Without fertilizers (control)	0-30	67.3	57.0	-10.3
	30-50	59.6	43.6	-16.0
2. N20	0-30	69.7	56.8	-12.9
	30-50	57.3	41.5	-15.8
3. N20P20S13	0-30	70.3	85.0	17.7
	30-50	68.0	59.9	-8.1
4. N20P25K25S23	0-30	68.0	87.9	19.9
	30-50	63.7	53.0	-10.7
NSR05	0-30			3.1
NSR05	30-50			2.9

The data obtained in 2019 confirm the decrease in the content of mobile phosphorus in the variant without fertilizers both in the 0-30 cm layer and in the 30-50 cm layer. In the variant with ammonium nitrate, the decrease in mobile phosphorus was 12.9 mg/kg in layer 0 -30 cm and 15.8 mg/kg in a layer of 30-50 cm. The use of phosphorus-containing fertilizers sulfoammophos and ammophos increased its content in the layer 0-30 cm by 17.7-19.9 mg/kg, and in the layer 30-50 cm, a decrease of 8.1-10.7 mg/kg was observed, which can be explained by the low mobility of phosphorus compounds and weak migration along the soil profile. An increase in the phosphorus content was observed only in the variant with the use of phosphorus-containing fertilizers in the 0-30 cm layer. In the 30-50 cm layer, its decrease is

observed in all variants, which will further lead to a clear differentiation of its content along the soil profile.

The content of mobile potassium in the soil after a seven-year period of introduction of No-till technology has decreased in almost all variants, with the exception of the variant with the use of potassium-containing fertilizer ammophoska. In these variants, the decrease was from 9.5 without fertilizers to 17.8 mg/kg in the layer of 0-30 cm and from 13.1 to 18.0 mg/kg in the layer of 30-50 cm. On the variant with the use of ammophos in the layer 0-30 cm, an increase in mobile potassium was observed by 12.3 mg/kg, and in the layer 30-50 cm by 3.7 mg/kg of soil (Table 3). On options without the use of potassium-containing fertilizers, seven years after the introduction of no-till

technology, a decrease in its content is observed both in the 0-30 cm layer and in the

30-50 cm layer, while no clear differentiation was observed.

Table 3. Change in the content of mobile potassium in leached chernozem in No-till technology

Option	Layer soil, cm	Mobile potassium, mg/kg soil		
		2013 (first year of implementation)	December 2019	Deviations from the original data
1. Without fertilizers (control)	0-30	136.0	126.5	-9.5
	30-50	127.1	114.0	-13.1
2. N20	0-30	128.3	110.5	-17.8
	30-50	125.3	109.0	-16.3
3. N20P20S13	0-30	120.0	107.0	-13.0
	30-50	120.0	102.0	-18.0
4. N20P25K25S23	0-30	133.8	146.1	12.3
	30-50	108.8	112.5	3.7
NSR05	0-30			2.2
NSR05	30-50			1.4

Table 4. Changes in pH(KCl) of leached chernozem in No-till technology

Option	Soil layer, cm	pH(KCl), units		
		2013 (first year of implementation)	December 2019	Deviations from the original data
1. Without fertilizers (control)	0-30	5.09	5.01	-0.09
	30-50	5.13	5.10	-0.03
2. N20	0-30	5.06	4.75	-0.31
	30-50	5.17	5.15	-0.02
3. N20P20S13	0-30	4.93	4.81	-0.12
	30-50	5.10	5.03	-0.07
4. N20P25K25S23	0-30	5.05	4.95	-0.10
	30-50	5.04	5.06	0.02
NSR05	0-30			0.07
NSR05	30-50			0.03

Determination of soil acidity is one of the most common analyzes, both in theoretical and applied research.

Plants show varying sensitivity to acidic and alkaline environments. The negative effect of acidity is especially dangerous in the initial growing season.

Studies have shown that the use of No-till technology reduces the processes associated with soil acidification.

The use of the soil without fertilization practically did not lower the pH level.

The use of complex fertilizers such as sulfoammophos and ammofoska reduced the pH by 0.10-0.12 units. in the 0-30 cm layer.

In the variant with the use of ammonium nitrate, an increase in acidity is observed in the 0-30 cm layer by 0.31 units. pH. In a layer of 30-50 cm, changes in pH values were within the experimental error.

## CONCLUSIONS

Studies have shown that the use of No-till technology without fertilizers for seven years led to a decrease in the content of alkaline hydrolyzable nitrogen in the leached chernozem in the upper thirty centimeter layer by 13.7 mg / kg of soil, mobile phosphorus by 10.3 mg / kg, and mobile phosphorus by 10.3 mg / kg. potassium per 9.5 mg / kg of soil. The introduction of mineral fertilizers in the form of ammonium nitrate, sulfoammophos and ammofoska increased the content of alkaline hydrolyzable nitrogen in the 0-30 cm layer by 12.1-13.8 mg / kg. The content of mobile phosphorus increased only in the 0-30 cm layer in the variants with the use of phosphorus-containing fertilizers by 17.7-19.9 mg / kg, and the content of mobile potassium in the variant with the potassium-containing fertilizer by 12.3

mg / kg of soil. Over the seven years of using fertilizers in No-till technology, there has been a tendency for a clear differentiation of the content of alkaline hydrolyzable nitrogen in soil layers of 0-30 and 30-50 cm when using nitrogen-containing fertilizers. According to the phosphorus content, differentiation according to the studied layers is manifested when using phosphorus-containing fertilizers. In terms of potassium content, no clear differentiation was observed in layers of 0-30 and 30-50 cm over the seven years of research.

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## RESEARCH ON SOIL FERTILITY IN THE EXPERIMENTAL FIELD AT THE MOARA DOMNEASCA EXPERIMENTAL STATION

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### **Abstract**

*A basic requirement in the development of modern fruit growing, that should be characterized by economic and ecological efficiency, is the detailed knowledge of the factors which are influencing the crop development, namely: environmental conditions and culture methods. Both aspects must have as final goal the maintenance and permanent improvement of the soil fertility. The rational, economically and ecologically efficient application of fertilizers can be achieved only by knowing in detail the physico-chemical conditions of the soil, considered as a means of living production necessary for plant growth. Thus we can determine the technological links in which we must intervene to obtain sustainable production both in terms of environmental protection and economically. It is also important to remember that by knowing these characteristics, we can identify the ecological methods of disease and pest control, given that the health of plants is directly influenced by the health and fertility of the soil. In 2019, before planting, soil samples were collected from depths of 0-20 cm and 20-40 cm. The samples were analyzed from the point of view of the main soil fertility indicators, respectively: pH, humus content, degree of saturation in bases, nitrogen index, mobile phosphorus, sum of bases and hydrolytic acidity, in order to determine the favorable cultivation of fruiting shrubs. The paper makes recommendations on the need to apply fertilizers in order to optimize the conditions for setting up a new orchard in an intensive system.*

**Key words:** substrate, humus content, nitrogen index, fertilizers, orchard.

### **INTRODUCTION**

The soil is an essential resource for all cultivated plants, being not only a support for plant roots, but also a reservoir of essential nutrients needed for plant growth. Due to the increasing practice of intensive agriculture, the soil is threatened by a number of factors, such as: erosion, loss of nutrient reserves, pollution, aridization, decreased fertility, etc. Overall, soil fertility has declined in the last fifty years, not only in our country but also worldwide.

Romania's geographical position and climate have always offered particularly favorable conditions for the cultivation of fruit trees and shrubs (Branîște, 2000). In this regard, Romania brings its contribution to the European Union through a valuable heritage, with many species of plants and animals, some endemic and relics, including medicinal plants and those with high sanogenic and nutritional value.

In this context, lately the attention of researchers and growers has increased towards

these species (Jerecki, 2012), registering an expansion of cultivated areas and an increase in fruit production in fruit bushes.

The humanity is facing a global process in terms of environmental protection, namely Climate change. The effects of these changes are already being felt. These changes have already had a considerable impact on ecosystems, the economy, and human health, as well as on well-being in Europe (according to the report "Climate change, impacts and vulnerability in Europe 2016").

The global and European temperatures reach new highs, at the same time the rainfall regime is changing, by increasing rainfall in the wetlands and the decreasing of rainfall in the arid regions.

At the same time, extreme climatic phenomena (heat waves, heavy rainfall, and periods of drought) are increasing in frequency and intensity in many regions of Europe, including Romania. All countries are vulnerable to climate change, but some regions are more exposed than others to the negative effect, with

most regions and sectors experiencing a negative impact.

In the climate of the Moara Domneasca Research Base, water losses through plant perspiration and evaporation from the soil are high. These losses are also influenced by strong winds that increase soil erosion. The organic matter content of the soil is generally low because the biomass production is low, which means that the availability of plant nutrients is very low. Under these conditions, the key to increasing crop productivity is to protect the soil from the sun, wind and increase the amount of organic matter and water in the soil. The organic matter content of the soil can be improved by applying manure or compost. In the case of compost application, the challenge is to increase the production of plant biomass, which is needed for compost production. In hot and humid climates, high above-ground biomass production and rapid decomposition of soil organic matter make nutrients easily accessible to plants. But it also involves a high risk of nutrients loss by being washed away and thus lost. Under these conditions, it is important to keep a balance between the production and decomposition of organic matter, so soil depletion is avoided.

The interaction between soil chemical, physical and microbiological properties define a specific soil's "quality" and influence how effectively the soil carries out ecosystem function such as: a) retain and release nutrients and other chemicals, b) distribute rainfall at the soil surface into run off and infiltration, c) hold and release soil water to plants, stream and groundwater, d) with stand wind and water erosion, and e) buffer against the concentration of potentially toxic materials (Pryce, 1991).

In the present research, the effects of the application of different technologies for cultivating fruit shrubs are studied (blackberry and raspberry), respectively planting on different culture substrates (compost used by mushrooms, mulberry, forest compost, etc.).

The most important to study as a culture substrate is the used compost of mushrooms, which is in fact the material left after finishing a mushroom culture. Used mushroom substrate (SMS) can be used in a wide variety of applications SMS is available in huge quantities underlined by the fact that 1 kg of

fresh mushrooms results in 5 kg of substrate consumed (ie 2 kg dry weight) (Finney et al. 2009). SMS has long been considered a waste stream. However, it can be used for production to produce high quality compost (Uzun, 2004) or other fungi to feed animals and for their health (Nasehi et al., 2017), to streamline the production of biofuels (Phan and Sabaratnam 2012), to produce materials (Jones et al., 2017; Islam et al., 2017; Appels et al., 2018), and to extract enzymes for industries (Phan and Sabaratnam, 2012).

The studied territory is part of the Moara Domneasca Farm, located N-E of Bucharest (in the Vlasiei Plain, a subunit of the Roman Plain), in Ilfov County at approx. 17 km from Bucharest. The farm belongs to the Fruit Research and Development Station Baneasa and is characterized by a humid climate, with hot summers and harsh winters.

Extreme temperature values range from 41.1°C in August to -30°C in January. The average annual rainfall is 580 mm, with a maximum average reached in June (92 mm) and a minimum average reached in February (31 mm).

The climate, the depth of the groundwater, the loessoid deposits and the vegetation contributed to the formation of soils belonging to the luvisols class, the reddish luvisols being predominant. In the depressed areas and in the crevices there are reddish luvisols and stagnosols.

The reddish luvisol from the Moara Domneasca, has the following characteristics:

- granulometric analysis for determination the soil content in clay, dust and sand revealed a high percentage of clay ranging from 40.5% in the upper horizon 0-40 cm, 41.6% at a depth of 41-53 cm and 47.4% at depths greater than 54 cm;
- the clay-clay texture determines a low mobility of nutrients and a poor permeability of water in the soil;
- apparent density on depth 0-20 cm 1.53 g/cm<sup>3</sup> medium, high at 20-40 cm 1.50 g/cm<sup>3</sup>.
- low total porosity over the entire depth of the profile.
- the degree of compaction has values between 16%, the soil is moderately compacted

and 18% at a depth of more than 40 cm, strongly compacted.

The morphological characteristics of the soil are:

***In the horizon of humus accumulation***

- Ao 0-19 cm, dusty clay, colors of 10YR 5/3.5 in dry state, loamy-clayey, poorly developed grainy-glomerular structure, (weak) - moderately compact, cracks to the base of the horizon, fine grassy roots, with rare point separations of iron and manganese hydroxides, gradual transition.

- Ao 20-40 cm, dark brown, with a slight reddish tinge, loamy-clayey, macro-microfuous, kneaded, angular, stable, loose, rare grassy roots, light, compact, with separations of iron hydroxide and manganese in the form of dots and spots.

***In the transition horizon***

- Ao / Bt 41-53 cm, clayey clay, dry colors 10 YR 4/3, grainy and small polyhedral angular and subangular, jilav, compact, with more separations of iron and manganese hydroxide more numerous, the transition is made gradually textural horizon B.

***On the horizon of colloquial illuviation***

- Bt 54-83 cm, reddish-brown, clay-clay, compact structure, shades of 10 YR 3/3.5, the transition from one sub-horizon to another is done gradually.

- Bt 84-125 cm clay clay, the shade of the material becomes reddish-reddish (5YR 3.5/4) in undisturbed structure.

- Bt 126-154 cm, clayey clay, prismatic-columnoid, very compact.

- Bt 155-200 cm clay clay, 5YR 4/4 in undisturbed structure and 5YR 4/4.6 in cut, compact, clear passage.

- About 200 cm depth, clay and loessoid clay material, under 2 m depth appear flowers and concretions of CaCO<sub>3</sub>.

## **MATERIALS AND METHODS**

At the Experimental Base Moara Domneasca, an agrochemical study of the soil at depth of 0-40 cm was carried out, in order to observe the main agrochemical soil fertility indicators which are directly influencing the quantity and quality of crops.

In 2019, after plots were plowed and harrowed, samples were taken by the personnel of the

Agrochemistry Laboratory. The samples were taken from plots that are to be planted with fruiting shrubs (different varieties of raspberries and blackberries). Sampling was done with a soil sampler, observing the provisions of the STAS 7184/1-84.

The samples were brought to the laboratory under normal conditions and processed according to the standard STAS 7184 .

The samples collected in the field phase were analyzed, according to the "Methodology for elaboration of pedological studies" - I.C.P.A, in the Agrochemistry Laboratory within the Fruit Research and Development Station Baneasa, using the following methods:

- soil reaction - pH - SR 7184-13 was determined in aqueous extract, soil: water ratio = 1: 2.5, with the CONSORT 933 analyzer using the pH electrode;

- humus content. STAS 7184/21-82 humus was determined by wet oxidation, according to the Walkley Black-Donut method, and titrimetric dosing;

- degree of saturation in bases - V% - STAS 7184/12-88;

- nitrogen index - IN =  $H \times V_{ah} / 100$  - STAS 7184/12-88;

- mobile phosphorus (assimilable) - Pppm - STAS 7184/19-82 were determined colorimetrically easily soluble phosphates in ammonium lactate acetate extract by the Egner-Riehm method, the values being read at UV spectrophotometer VIS CAMSPEC M 330;

- sum of bases - SB -. STAS 7184/12-88;

- hydrolytic acidity - Ah.

The determination of the hydrolytic acidity and the sum of the exchangeable bases was performed according to the Kappen method. In order to correct the soil reaction, the degree of base saturation, VAh, was determined, which represents the ratio between the sum of the basic cations, SB, and the total cation exchange capacity, SB + Ah. To estimate the amount of mineralized organic matter in the soil and the amount of mineralized N in it, the nitrogen index was determined,  $IN = H * VAh/100$ .

After determining the values of agrochemical indicators, it can be identified the ecological methods to control diseases and pests, given that plant health is directly influenced by soil health and fertility.

The basic fertilization must take into account the results of the agrochemical analysis and bring a contribution in macroelements balanced with the needs of the species. Any supply of organic matter at orchard establishment will bring positive impact on plants growing rate. Also, an organic fertilizer will improve the soil structure and texture and will actively support the microbiological life of the soil surface. Thus, we developed a experiment in which different organic substrates have been used in order to identify which of these are more beneficial for the cultivation of fruit bushes.

## RESULTS AND DISCUSSIONS

In the Moara Domneasca soil profile there are biogenic neoformations, especially in the upper part of the profile (coprolites, larval sites, etc.), and iron oxides and hydroxides are on the crack walls like films, usually continuous in the Bt horizon. In horizon C we find neoformations of calcium carbonate. The granulometric composition of the soil shows that the clay content increases from the soil surface to the Bt horizon, from 40.55% to 47.39%, and the fine sand decreases with depth (Table 1).

Table 1. The granulometric composition of the reddish luvisols soil at the Moara Domneasca

Horizon	Depth cm	Clay %	Coarse sand %	Fine sand %	Dust %	Texture
Ao	0-40	40.55	0.36	34.33	24.75	Clay loam
Ao/Bt	41-53	41.63	0.52	21.54	56.28	Clay loam
Bt	54-200	47.39	0.37	27.59	30.34	Clay loam
C	Over 200	36.18	0.42	32.04	32.04	Clay loam

The soil content in humus is good in the first 40 cm of profile, where most of the roots of young trees are located, reaching 3.26%, then suddenly decreases in profile up to 1% in the

Bt horizon (Table 2). The pH is slightly acidic at the soil surface (6.4), reaching alkaline in the C horizon (8.3).

Table 2. Physico-chemical properties of reddish luvisols soil at the Moara Domneasca (on profile)

Factor	Ao	Ao/Bt	Bt	C
Humus (%)	3.26	1.87	1.0	1.0
Ca soluble (mg/100 g sol)	55	32	32	30
Hydrolytic acidity(m.e.)	2.8	2.04	1.72	0.18
Amount of exchangeable bases (m.e.)	22.6	23.62	26.28	-
Total cation exchange capacity (m.e.)	28.65	28.04	30.01	-
Degree of saturation in bases (%)	78.94	84.28	87.53	-
pH	6.4	6.6	6.8	8.3
Total nitrogen (%)	0.144	0.102	0.075	0.07
Soluble phosphorus (mg/100 g sol)	50	40	40	30
Soluble potassium (mg/100 g sol)	2	2	2	2

### Soil preparation for planting

Table 3. The results of the soil samples at the Moara Domneasca, 2019

Date	2019		
	Lot no.	SHRUBS (plowed soil)	
		Depth	
		0-20	
pH	6.26	6.39	6.35
SB: Ca(2+)+Mg(2+)+K(+)+Na(+)	7.94	15.68	16.27
Ah	6.17	6.17	4.46
H, % (105 C)	3.14	2.94	3.02
C org (105 C)	5.42	5.08	5.21
T - cation exchange capacity	14.11	21.85	20.73
Degree of saturation with bases (%)	56.26	71.75	78.49
IN (105 C)	1.77	2.11	2.37
P, mg/kg	50.45	56.57	46.86

After planting, before substrate application, the results of soil analysis were:

Table 4. The results of the soil samples at the Moara Domneasca before substrate application, 2020

Date/lot no.	FEBRUARY 2020				Normal values allowed by Order no. 756/1997	
	raspberry		blackberry		raspberry	blackberry
Sample no.	13	14	15	16		
Depth	0-20	20-40	0-20	20-40		
pH	7.07	6.74	7.3	6.99	5.6-6.5	5.8-7.0
U, % humidity	18.37	15.71	18.15	17.12		
SB: Ca(2+)+Mg(2+)+K(+)+Na(+)	12.23	12.15	12.29	12.2		
Ah	2.74	2.56	2.77	2.34		
H, % (105 C)	4.1	2.47	4.46	3.98	3.5-4.5	3.5-4.5
C org (105 C)	2.38	1.43	2.59	2.31		
T - cation exchange capacity	21.34	23.56	23.57	20.34		15
V <sub>Ab</sub> , % - degree of saturation with bases	98.37	98.71	98.35	99.23	76-92	80-98
IN (105 C)	4.03	2.44	4.39	3.95	4.1-6	4.1-6
P, mg/kg	101.9	62.6	60.1	88.4	>72	>72

## Experimental design

In March 2020, the most appropriate options for these crops were fixed.

The randomized experimental design was Randomized Complete Block with 2 species (blackberry and raspberry) on different variants of organic substrate (V1-garden soil, V2-used mushroom compost, V3-forest compost, V4-mixture V1 + V2 + V3 + V5, V5-semi-fermented compound and M-control).

Planting distance between rows - 3m and planting distance between plants (blackberry - 1m and raspberry - 0.5 m).

As varieties, for blackberries, have been used Triple Crown, Chester and Dar 24.



Figure 1. Planting the plants



Figure 2. Soil substrate

As varieties, for raspberries, have been used Tulameen, Przehyba and Citria.

In order to supply plants with water a drip irrigation system was used. For weed protection mulching has been done with Agrotexile foil.

For each substrate variant I will use 7 blackberry plants of each variety and 7 plants/variety as control and 14 raspberry plants of each variety and 14 plants/variety as control.

## CONCLUSIONS

The results of the analyzes show that the reddish preluvosol from Moara Domneasca on which the experimental lots of fruit bushes are placed is well provided with nutrients.

The humus content includes this soil in the category of medium soils provided with humus, and phosphorus is also found in the category of medium soil provided with this element.

In order to increase the fertility of this soil, we cultivated fruit bushes on different cultivation substrates (used compost from mushrooms, garden soil, forest compost, semi-fermented compost).

Determinations will be made in plants throughout the vegetation periods to highlight the influence of nutrient substrates on the ripening date of the first / last fruits in the inflorescences and on the growth and fruiting processes.

The aim of the experiment is to apply cultivation technologies targeting reducing soil degradation, by optimizing agrochemical soil properties, maintaining and increasing soil nutritional capacity by using technologies for the balanced application of fertilizers (organic and mineral).

In the given context, the elaboration of soil fertilization recommendations should be based on periodic control of soil fertility status depending on:

- Cropping system, assortment and applied technology;
- Soil maintenance system;
- Biological particularities of the species
- Irrigation method used;
- Integrated control of diseases and pests.

Depending on these considerations, the soil fertilization of experimental plots, must include recommendations for the combined and harmonized use of all nutrient resources (organic, mineral and foliar fertilizers).

The growths that take place will continue to be monitored, depending on the substrate in which the fruit bushes are grown and the variation of the nutrients in the soil in each substrate.

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## AMPLIFICATION OF COMPACTION AND SALINIZATION OF POLYTUNNELS SOILS WITH COARSE TEXTURE AFTER MULCHING WITH PLASTIC FOIL

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### Abstract

*In Romania, the surfaces of solariums are extended significant in the last period due to high quality of polythene film used to cover greenhouses and due to improving of plants growing technology such as using the best varieties, plastic mulch and drip irrigation. Our studies performed includes determination of the main morphological parameters, penetration resistance, soil sampling in the field and soil analysis (soluble salts, bulk density, size particles, pH, calcium carbonate) in the laboratory. Following the investigations, it was found that mulching with black plastic foil on the entire surface favors soil compaction and local accumulation of soluble salts especially in the marginal area of the wetting front resulting from drip irrigation. The accumulation of soluble salts was also noticed in the superficial layer of soil on the interval between rows of plants. Wetting of the soils with coarse texture on the intervals between the rows of plants took place following the evaporation, diffusion and condensation of water vapor in the space between the foil and the soil surface. Strong soil compaction and salinization allows us to recommend avoiding plastic mulch over the entire soil surface.*

**Key words:** plastic foil, coarse texture, penetration resistance, compaction, salinization.

### INTRODUCTION

High tunnels have become a popular and profitable method to produce high-value crops. They are simple, tall, plastic-covered structures used for the production of fruits and vegetables, cut flowers, and many other crops. They resemble greenhouses but cost less to erect and operate (William & Lamont, 2009).

Growing vegetables in plastic tunnels has greatly expanded in Romania due to the significant advantages compare with greenhouses covered with glass. In temperate zones some of glass used to cover greenhouses breaks in cold season (due to strong frost) and in the warm season under influence of hail.

The main criterion considered by farmers in order to choose the location for plastic tunnels is the existence of a good quality water source to meet the requirements of plants grown throughout the growing season during the year. The location of plastic tunnels near the market in order to diminish the transport costs is another main criterion.

Due to the compulsory location imposed by the above conditions, many plastic tunnels are

placed on soils considered with a low capability but then through the application of land improvement works satisfactory results have obtained (Canarache, 1995).

The soils of first capability class for plastic tunnels location must have a loamy sand texture (clay- 12-20%) without coarse rock fragments or artefacts (i), large reserve of humus, calcium carbonate content less than 4%, good water and air permeability, good lateral drainage of groundwater, slightly acidic or neutral or slightly alkaline reaction, small content of soluble salts and exchangeable sodium (Florea et al., 1997; Canarache, 1973).

The high soil moisture in plastic tunnels, high values of temperature during the year favors the activity of microorganisms in the organic matter decaying. After this process result high quantities of CO<sub>2</sub>. The absence of air currents which assure the change of soil air lead to the necessity of soil air porosity value higher than 10% (v/v), value of which represents the minimum limit of air content for field soils (Filipov et al., 2002).

Interest concerning to processes of plastic tunnels soil degradation developed nowadays,

mainly due intensive agriculture management system on lower quality soils and increasing in technological inputs. Various soil degradation processes are considered among them salinization, sodization, crusting, compaction, (Canarache, 1991; Maianu, 1974, Filipov et al., 2004; Filipov & Topa, 2020).

The accumulation of a high content of soluble salts (after the mineralization of the organic material or after irrigation with water containing soluble salts) also restricts the plants growth due to direct ion toxicities (e.g. sodium, chloride, boron, etc.), ionic imbalance of the plants and decreasing of the water availability (physiological drought). The plant growths rate is influenced by the effect of salts on leaf area per plant, on leaf thickness, on daily increment in thickness and area of primary leaves, on the changes in the amount of vascular tissue into stem and other effects (Maianu, 1974; Borlan & Hera, 1984).

Soil with coarse texture (clay <12%) are known under the name of Psamosol (Florea & Munteanu, 2012) and belong to loamy texture class or coarser. These soils have low water-holding capacities, high water permeability, excessive aeration, low content of all essential nutrients, low buffer capacity and are still much less fertile than most soils in the regions they are located in. The sandy or loamy sand soil are susceptible to physical degradation by wind erosion and compaction processes. Soil vulnerability to compaction increase if the fine sand/coarse sand ratio is lower than 10. Following compaction, the permeability of coarsely textured soils decreases and the accumulation of soluble salts is amplified.

## MATERIALS AND METHODS

The study site was located at poly-tunnels Pahnesti, located in the northwestern part of the Husi Depression and at the eastern edge of the Central Moldavian Plateau.

Average altitude of Pahnesti is 132 m. The village is drained by Pahnesti river with an intermittent discharge.

Some soil profiles were made inside of studied plastic tunnels. These profiles were morphologically described according to the Methodology of soil survey elaborated by the

Research Institute for Soil Science and Agrochemistry, Bucharest [12].

After morphological soil description in the field, undisturbed samples from 10 to 10 cm were collected down to, the depth of 50 cm. The bulk density was determined in the laboratory (Figure 1).



Figure 1. Sampling of soil in stainless steel cylinders to assess the state of soil compactness - three replicates

In the field, also was determined the penetration resistance (Figure 2) of the soils by using a digital penetrometer (Eijkelkamp Equipment, Model 0615-01 Eijkelkamp, Giesbeek, The Netherlands) which had a cone angle of 30° and a base area of 1 cm<sup>2</sup>.



Figure 2 Determination of penetration resistance in Pahnesti plastic tunnel

It was carefully inserted into the soil profiles in 1 cm increments from the surface to a depth of 80 cm. Ten parallel records were made in each plot and averaged for analysis.

Disturbed samples from the soil profiles were also taken. In the laboratory conduct size particles analyses, pH, bulk density, content of water-according to the current methodology (Dumitru et al., 2009; Lăcătușu et al., 2017).

The period of our investigations of polytunnels soils with coarse texture from Husi Depression was 2019-2020.

Following the processing and analysis of the data obtained in the field and laboratory, several reclamation measures have been recommended.

## RESULTS AND DISCUSSIONS

After Romanian System of Soil Taxonomy (Florea & Munteanu, 2012) the studied soil have been diagnosed as Hipohortic Salinic Gleic Aluviosols (Figure 3A).

The studied soil has a coarse texture.

Following studies of the soil profiles, the presence of the plough pan (Figure 3A) was noticed, which we consider to be the cumulative effect of agricultural technologies practiced until and after the establishment of plastic tunnels.

The higher frequency of the roots on the faces of the structural elements within the plough pan is obvious (Figure 3B).

In the lower part of the profile there is the presence of a compact lithological layer with obvious reductomorphic characteristics due to the groundwater infiltration from the Pahnesti river (Figure 4).

Hipohortic Salinic Gleic Aluviosols has an uneven texture, the loamy sand horizons alternate with those with sandy loam layer (Tabele 1).



Figure 3. A- Soil profile of Hipohortic Salinic Gleic Aluviosols; B- Preferential distribution of roots on the faces of structural elements within the plough pan layer



Figure 4. Details with reductomorphic features on the lower part of soil profile

Table 1. Present moisture content, texture and state of soil compactness

Depth cm	Wg %	BD g cm <sup>-3</sup>	Wv %v/v	Texture	State of compactness
0-10	17.3	1.1	19.03	loamy sand	very loose
10-20	21.8	1.15	25.07	loamy sand	very loose
20-30	19.9	1.33	26.47	loamy sand	loose
30-40	19.5	1.55	30.22	loamy sand	slight compacted
40-50	18.8	1.52	28.57	sandy loam	slight compacted
50-60	18.5	1.61	29.79	loamy sand	slight-mod. compacted

BD - bulk density; Wg- gravimetric water content; Wv -volumetric water content

The higher value of bulk density of 1.61 g/cm<sup>3</sup> is recorded in the lower part of the soil profile. The slight to moderate compaction of this soil

layer is due to the previous alluvial processes and not to the current pedoecetic processes. The lowest value of soil density of  $1.1 \text{ g/cm}^3$  is in the hortic horizon (Aho) and is due to the high content of organic matter. It is noted that the highest value ( $30.22\%v/v$ ) of the gravimetric water content corresponds to the plough pan horizon. Higher compactness state of plough pan favours water stagnation.

The slight compaction of the soil is also highlighted by the higher values ( $1.8\div 2.0 \text{ MPa}$ ) of the penetration resistance recorded in the plough pan (Figure 2). The more intense compaction of the under ploughed horizon favours the accumulation of a larger quantity of soluble salts in the ploughed horizon (Figure 6).

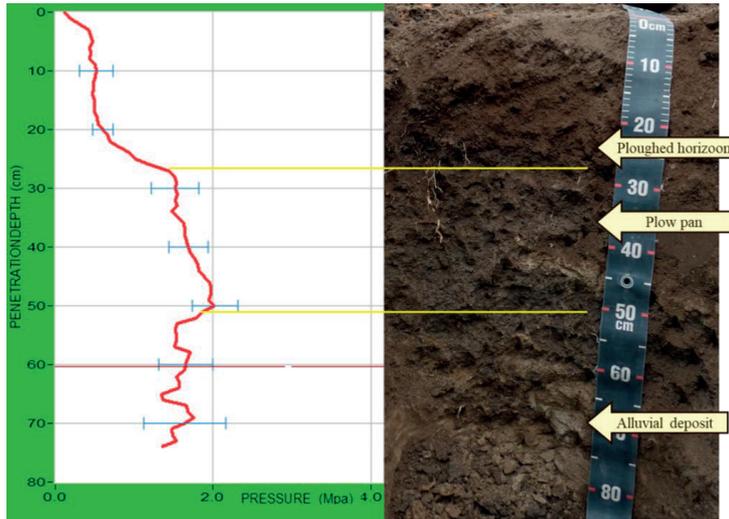


Figure 5. The presence of the ploughpan highlighted by penetration resistance value on the hipohortic stagnic salinic Aluviosol



Figure 6. The more intense compaction of the under ploughed layer determines the extension of the wetting strips and the accumulation of soluble salts (A-10 replicates of penetration resistance determination; B- accumulation of soluble salts; The 10 repetitions of determining the resistance to penetration)

The soil contains calcium carbonate in all pedogenetic horizons. Calcium carbonate gives the soil a slightly alkaline reaction with pH values between 7.65 and 8.47 (Table 2).

The high humus content in the plough layer is due to the application of high doses of organic fertilizers represented by old manure.

High contents of organic matter (5.21%) and P<sub>2</sub>O<sub>5</sub> (>250ppm) are defining horticultural horizons.

Hypohorticultural stagnic saline Aluvisol it is well supplied with nitrogen, phosphorus and potassium.

Table 2. Some chemical characteristics of hypohorticultural stagnic saline Aluvisol

Depth cm	Soil horiz.	pH	CaCO <sub>3</sub> %	OC %	Nt %	P <sub>2</sub> O <sub>5</sub> ppm
0-20	Apsc	7.7	2.7	3.1	0.32	431
20-35	Atp sc	8.1	7.0	0.87	0.08	82
50-60	AC sc	8.2	7.1	0.77	0.07	56
60-100	AGosc	8.5	8.6	-	-	-

Soil horizon- symbols of soil horizons; OC – Organic Carbon; Nt-total Nitrogen; ppm mg/kg.

The main limiting factor of the studied soil fertility is the high content of soluble salts. The maximum accumulation of soluble salts of 752 g/100g is recorded in the upper part of the soil (Table 3).

Table 3. Content of anions and soluble salts (ss) of hypohorticultural stagnic saline Aluvisol (mg/100g soil)

Depth cm	Cl <sup>-</sup>	SO <sub>4</sub> <sup>-</sup>	HCO <sub>3</sub> <sup>-</sup>	meCl/meSO <sub>4</sub> <sup>-</sup>	ss
0-20	178	279	59.8	0.86	752
20-35	184	86	47.8	2.89	464
50-60	134	40	35.9	3.2	306
60-100	48	10	23.7	6.75	11

We consider that the salts from the horticultural horizon come from the waters used for irrigation, the decomposition of the organic matter and from the phreatic water coming from the lateral infiltrations from Pahnesti River.

The dominant salts in the soil are sodium chloride and sodium sulphate.

Strong chloride salinization of the soil is highlighted by the high content of chlorine recorded in the upper part of the soil from the depth range 0-60 cm.

It is known that chlorine has a greater negative influence on plants than the SO<sub>4</sub> anion.

Among vegetables commonly grown in plastic tunnels, the highest sensitivity to chlorine have plants from *Solanaceae* family (Sala, 2017).

The lowest value of ratio Cl<sup>-</sup>/SO<sub>4</sub><sup>2-</sup> recorded in the ploughed horizon suggests that the soluble salts also come from another source than that of irrigation water.

The main source for the bicarbonate (HCO<sub>3</sub><sup>-</sup>) anion is calcium carbonate present in all soil horizons.

The irrigation water contains 420 mg/dm<sup>3</sup> soluble salts. The value of ratio Cl<sup>-</sup>/SO<sub>4</sub><sup>2-</sup> recorded in irrigation waters is 1.4. The high magnesium content is noticeable in the saline composition of the irrigation water (Table 4).

Table 4. Chemical composition of water irrigation (mg/dm<sup>3</sup>)

Cl <sup>-</sup>	SO <sub>4</sub> <sup>-</sup>	HCO <sub>3</sub> <sup>-</sup>	Na <sup>+</sup>	K <sup>+</sup>	Ca <sup>++</sup>	Mg <sup>++</sup>
92.7	93.1	111.6	15	4	32	51.1

This composition of water may be due to anthropogenic influence. The magnesium content in municipal waters is frequently higher than in groundwater or rivers.

It is necessary to extend the range of analyzes performed in the lab in order to assess the quality of irrigation water. Given that MgCl<sub>2</sub> and MgSO<sub>4</sub> are toxic salts, we recommend caution in using these waters for irrigation. Lixandru (1991) noticed that magnesium ion associated with exchangeable sodium greatly worsens soil properties.

Given that MgCl<sub>2</sub> and MgSO<sub>4</sub> are toxic salts, we recommend caution in using these waters for irrigation and repeated monitoring of water and soil quality.

Mulching with the plastic foil has also the negative effect of on the soil by increasing the water content resulting from the condensation of evaporated water from the irrigated soil surface, intensifying soil compaction, local accumulation of soluble salts especially in the border of wetted strip. The compact soil favours the accumulation of soluble salts.

## CONCLUSIONS

The studied soil has been diagnosed as Hypohorticultural Salinic Gleic Aluvisols with slight moderately ploughpan and obvious reductomorphic characteristics in the bottom part of profile.

Higher compactness state of ploughpan highlighted by 1.8÷2.0 MPa of the penetration

resistance favors water stagnation and salt accumulation in the ploughed horizon. High contents of organic matter and P<sub>2</sub>O<sub>5</sub> are defining hortic horizon which is well supplied with nitrogen, phosphorus and potassium. The main limiting factor of the studied soil fertility is strong chloride salinization of the upper part from the depth range 0-60 cm. The high magnesium content of water irrigation could be due to anthropogenic influence. Given that MgCl<sub>2</sub> and MgSO<sub>4</sub> are toxic salts, we recommend caution in using these waters for irrigation and repeated monitoring of water and soil quality. Mulching with the plastic foil has also the negative effect of on the soil by increasing the water content in soil not moistened with irrigation water, intensifying soil compaction, local accumulation of soluble salts especially in the border of wetted strip.

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## EVALUATION OF UNCERTAINTY FOR DETERMINATION OF IRON FROM ORGANO-MINERAL FERTILIZERS BY ATOMIC ABSORPTION SPECTROMETRY

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### Abstract

*Organo-mineral fertilizers trade has known a significant growth in the internal market. Also, the range of products in this category has greatly diversified in the last period. In order to ensure fair competition in the fertilizer market and to protect the interests of end - users, European Regulations imposed higher requirements regarding marketing and quality control of fertilizers. Thus, it is now a formal requirement for laboratories to introduce quality assurance measures to ensure that they are capable of and are providing data of the required quality. Such measures include: accreditation based on ISO 17025 or the use of validated methods of analysis. Among several parameters, evaluation of uncertainty plays an important role in method validation. This paper presents the evaluation of uncertainty for determination of iron in organo-mineral fertilizers by means of atomic absorption spectrometry. A complex organo-mineral fertilizer was used for analysis and uncertainty sources were identified and quantified for each step of the process: weighing, dilution, equipment calibration and measuring iron concentration. Relative expanded uncertainty was 9.62% and repeatability was the main component of the uncertainty budget.*

**Key words:** fertilizers, iron, uncertainty.

### INTRODUCTION

It is expected that in the next 40 years the demand for food will increase by over 60% as the global population will rise to 9.7 billion in 2050. The global population growth will increase the demand for agricultural and food production, thus leading to a rising dependence on fertilizer inputs. The technology progress around the world, which works with modern environmental issues, attracts attention to agriculture and makes the domain of fertilizers technology the cornerstone for industrial development. The fertilizer market was valued at USD 155.80 billion in 2019, and it is estimated to register a CAGR of 3.8% by 2025. In the last period, organic and organo-mineral fertilizers trade have known a significant growth in the international market and the range of products in this category has greatly diversified (Lee, 2011; Mordor Intelligence, 2020).

Organo-mineral fertilizer can be defined as "a fertilizer obtained by blending, chemical reaction, granulation or dissolution in water of inorganic fertilizers having a declarable content

of one or more primary nutrients with organic fertilizers or soil improver" (Antille et al., 2013).

The use of organo-mineral fertilizers in agriculture is in accordance with the concept of sustainable intensification of agriculture. The definition of "sustainable agriculture" originates back in the USA in the early 1980s, indicating a way of farming that should mimic natural ecosystems (Gomiero et al., 2011). In the last years, a concept called "sustainable intensification" has been discussed, meaning producing more food from the same area of land while reducing the environmental impacts (Godfray et al., 2010).

It is reported by various authors that organo-mineral fertilizers give similar or higher yield responses as compared to conventional fertilizers for diverse crops: wheat, maize, barley, oil seed rape, pepper, amaranthus (Ailincăi et al., 2008; Akanni et al., 2011; Ayeni et al., 2012; Deeks et al., 2013). One of the most important features of using organo-mineral fertilizers is the "slow release" effect: mineral components are protected by the

binding and absorption of organic components which results in a more gradual release of nutrient to the soil and the reduction of nutrient losses to the environment (Kominoko et al., 2017).

With Romania's admission to the European Union, the national legislation on the regulation of marketing and quality control of fertilizers has been aligned with Community legislation. The higher requirements imposed by European Regulations are intended to ensure fair competition in the fertilizer market, to protect the interests of end - users, as well as to reduce the negative effects of these products on the environment. Among the measures for national implementation of the provisions of EU regulations are those relating to the organization of quality control of fertilizers activities by authorized laboratories, accredited by the national accreditation body (RENAR) according to ISO 17025. Thus, the laboratories are formally required by the mentioned standard to introduce in their management system measures for quality assurance, by which to demonstrate that they are able to provide analytical results of the quality required by customers or regulatory requirements (Grigore et al., 2011).

The use of validated methods is important for an analytical laboratory to show its qualification and competency. Method validation is done by evaluating a series of method-performance characteristics, such as precision, trueness, selectivity/specificity, linearity, operating range, recovery, limit of detection (LOD), limit of quantification (LOQ), sensitivity, robustness, measurement uncertainty (Magnusson & Örnemark, 2014). Uncertainty is a parameter associated with the result of a measurement that characterises the dispersion of the values that could reasonably be attributed to the measurand (Ellison & Williams, 2012).

Measuring uncertainty is a comprehensive parameter covering all sources of error and thus more than method validation alone. In practice, data from method validation and collaborative studies form the basis for measuring uncertainty evaluation. An analytical result must always be accompanied by an uncertainty statement. The interpretation and the use of any measurement fully depend on the uncertainty (at a stated level of confidence) associated with it (Taverniers, 2004).

This paper describes a method to evaluate measurement uncertainty for iron determination in organo-mineral fertilizers by flame atomic absorption spectrometry technique.

## MATERIALS AND METHODS

### Reagents

All reagents used were supplied by Merck (Darmstadt, Germany) and were of analytical purity: hydrochloric acid 37% HCl,  $c(\text{HCl}) = 12 \text{ mol/l}$ , nitric acid 65%,  $c(\text{HNO}_3) = 14.3 \text{ mol/l}$ , and lanthanum chloride heptahydrate ( $\text{LaCl}_3 \cdot 7\text{H}_2\text{O}$ ). CertiPUR® standard solution 1000 mg/l Fe was used for calibration curve. Ultra-pure water was used to prepare all solutions.

### Equipment

Determination of iron was performed using an atomic absorption spectrometer (AAS) Thermo Electron iCE 3300 (UK) with a hollow cathode lamp as radiation source, a deuterium lamp as background correction and air-acetylene flame. The working parameters are listed in Table 1. Ultra-pure water was obtained using a water purification system Evoqua (Germany).

Table 1. Working parameters for AAS

Parameter	Iron (Fe)
Wave length (nm)	248.3
Lamp current (mA)	15
Acetylene/air flow (L/h)	1.2
Burner (mm)	100

### Procedure

A complex NPK organo-mineral fertilizer was used for analysis. 2 g of the sample were weighted and quantitatively transferred to the reaction vessels. The test portions were moistened with about 0.5 ml to 1 ml of water and then, while mixing, 14 ml of HCl 37% and 4.7 ml of HNO<sub>3</sub> 65% were added. The reaction vessels were placed to the heating device until complete mineralization. After cooling, samples were transferred quantitatively into a 100 ml volumetric flask and diluted to the mark with ultra-pure water. Test solutions were filtered using ash-free filter paper. 20 ml of the sample extract were pipetted into a 100 ml volumetric flask, then 10 ml of diluted HNO<sub>3</sub> (5 mol/L) and 10 ml of lanthanum solution (10 g/L) were added. The flasks were filled to the mark with ultra-pure water, mixed well and

the solutions used for measurement. A blank test solution was prepared following the same procedure as for sample.

#### Calibration curve

In order to obtain the stock solution 10 ml of standard were pipetted into a 100 ml volumetric flask. The flask was filled to the mark with ultra-pure water. Adequate aliquots of standard solution were diluted with nitric acid solution (0.5 mol/l) to obtain 6 concentrations levels (0.5; 1.0; 1.5; 2.0; 2.5, and 3.0 mg/L Fe) and a squared correlation coefficient  $r^2 > 0.9990$  was considered acceptable.

#### Evaluation of uncertainty

The steps involved in evaluation of uncertainty are: specifying the measurand, identifying the uncertainty sources, quantifying the uncertainty components, and calculating the combined uncertainty.

#### Identification of the uncertainty sources

The measurand is the concentration of iron extracted from the complex fertilizer according to the procedure shown above.

$$c(Fe) = \frac{c_0 \times V_{100} \times d \times 10^{-4}}{m_s} \times rep \quad (\%) \quad (1)$$

Where:

$c_0$  – concentration of iron in the aliquot solution (mg/l),

$V_{100}$  – volume of the extraction solution (ml),

$d$  – dilution factor,

$m_s$  – mass of the sample (g)

rep – repeatability (the repeatability estimate is treated as a relative effect; rep=1).

The relevant sources of uncertainty were identified by constructing a cause-and-effect diagram (Figure 1). The main cause branches represent the parameters controlling the result as shown in Eq. 1.

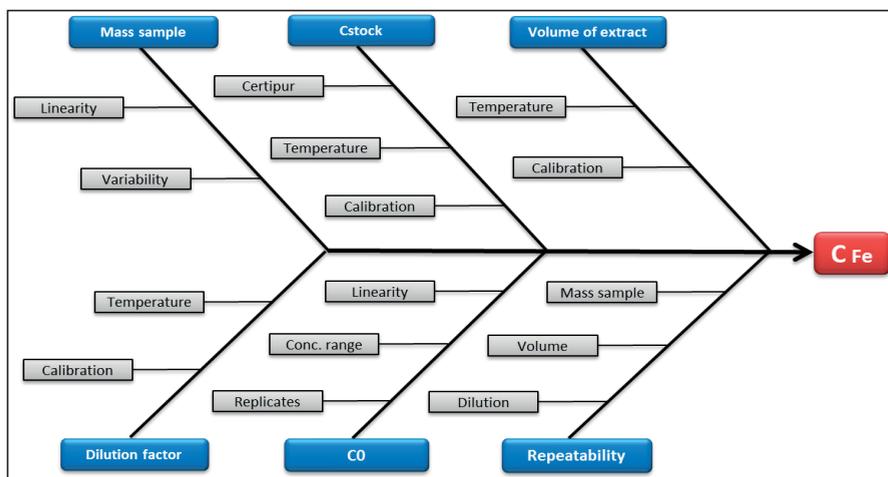


Figure 1. Cause-and-effect diagram for iron determination from organo-mineral fertilizers by AAS

## RESULTS AND DISCUSSIONS

#### Quantification of the uncertainty sources

Quantification of the uncertainty sources is done by using experimental data and standing data such as calibration certificates and reference materials certificates.

#### Mass of the sample, $m_s$

The uncertainty associated with the mass of organo-mineral fertilizer sample arises from the balance linearity and standard deviations of control charts (data obtained from verification of the balance using standard weights of 10 g and 50 g). The calibration certificate of the

balance quotes  $\pm 0.0003$  g for the linearity. Considering a rectangular distribution, the balance linearity contribution is 0.00017 g. The contribution from the balance linearity has to be counted twice, once for the tare and once for the gross weight, because each is an independent observation and the linearity effects are not correlated. The uncertainty associated with the mass of the sample is calculated accordingly:

$$u(m_s) = \sqrt{2 \times 0.00017^2 + 0.0002^2 + 0.0003^2}$$

$$\Rightarrow u(m_s) = 0.00044 \text{ g}$$

### Stock solution, $c_{stock}$

The uncertainty from stock solution depends upon the uncertainty of standard solution and volume. According to the supplier's certificate, concentration of the standard solution was  $984 \pm 4$  mg/kg. Assuming a normal distribution, the standard uncertainty of Certipur solution is  $u(c_{certipur}) = 2$  mg/kg.

The uncertainty associated with the volume has repeatability, calibration, and temperature as major influences. The repeatability is being taken into account via the combined repeatability term for the experiment. The calibration certificates quote volumes for the flask of  $100 \text{ ml} \pm 0.030 \text{ ml}$  and for the pipette of  $10 \text{ ml} \pm 0.023 \text{ ml}$  measured at a temperature of  $20^\circ\text{C}$ . The standard uncertainties are calculated assuming a normal distribution:  $u(V_{c100}) = 0.0150 \text{ ml}$  and  $u(V_{c10}) = 0.0115 \text{ ml}$ . According to the calibration certificates, the flask and the pipette have been calibrated at a temperature of  $20^\circ\text{C}$ , whereas the laboratory temperature varies between the limits of  $\pm 4^\circ\text{C}$ . The coefficient of volume expansion for water is  $2.1 \times 10^{-4} \text{ }^\circ\text{C}^{-1}$ , which leads to a volume variation of  $\pm 0.0840^\circ\text{C}$  and  $\pm 0.0084^\circ\text{C}$  respectively. The uncertainties are calculated using the assumption of a rectangular distribution for the temperature variation:  $u(V_{t100}) = 0.0489 \text{ ml}$  and  $u(V_{t10}) = 0.0049 \text{ ml}$ . The two contributions are combined to give the standard uncertainties of the volumes:  $u(V_{100}) = 0.0508 \text{ ml}$  and  $u(V_{10}) = 0.0124 \text{ ml}$ .

Combining the intermediate standard uncertainties above calculated, the standard uncertainty of the iron stock solution is given by Eq. 2.

$$\frac{u(c_{stock})}{c_{stock}} = \sqrt{\left(\frac{u(c_{certipur})}{c_{certipur}}\right)^2 + \left(\frac{u(V_{100})}{V_{100}}\right)^2 + \left(\frac{u(V_{10})}{V_{10}}\right)^2} \quad (2)$$

$$\frac{u(c_{stock})}{100} = \sqrt{\left(\frac{2}{984}\right)^2 + \left(\frac{0.0508}{100}\right)^2 + \left(\frac{0.0124}{10}\right)^2}$$

$$\Rightarrow u(c_{stock}) = 0.2437 \text{ mg/l}$$

### Dilution factor, $d$

The relative uncertainty from the dilution factor is  $u(d) = 0.00086$  and was calculated combining the relative uncertainties associated with the volume for the 100 ml flask and 20 ml pipette.

The calculation of the uncertainty associated with the volume is previously described.

### Concentration of the aliquot solution, $c_0$

The calibration curve was manually prepared. For this purpose, six calibration standards, with concentrations of 0.5 mg/L, 1.0 mg/L, 1.5 mg/L, 2.0 mg/L, 2.5 mg/L and 3.0 mg/L, were prepared from a 1000 mg/L iron reference standard. The usual uncertainty calculation procedures for  $c_0$  only reflect the uncertainty due to random variation in the absorbance and not the uncertainty of the calibration standards, nor the inevitable correlations induced by successive dilution from the same stock which are sufficiently small to be neglected (Ellison and Williams, 2012).

The six calibration standards were measured three times each providing the results in Table 2. The results of the linear least square fit are  $B_1 = 0.12347$ ,  $B_0 = 0.00927$  with a correlation coefficient of 0.9995. The sample solution was measured three times also, leading to a concentration  $c(0)$  of 1.53 mg/L.

Table 2. Calibration results

Concentration (mg/L)	Absorbance (replicates)		
	1	2	3
0.5	0.067	0.068	0.069
1.0	0.133	0.134	0.133
1.5	0.196	0.198	0.196
2.0	0.258	0.261	0.259
2.5	0.317	0.317	0.319
3.0	0.377	0.377	0.377

The uncertainty of iron concentration  $u(c_0)$  is given by Eq. 3, with the residual standard deviation  $S$ , given by Eq. 4 and  $S_{xx}$  given by Eq. 5.

$$u(c_0) = \frac{S}{B_1} \sqrt{\frac{1}{p} + \frac{1}{n} + \frac{(c_0 - \bar{c})^2}{S_{xx}}} \quad (3)$$

$$S = \sqrt{\frac{\sum_{j=1}^n [A_j - (B_0 + B_1 \times c_j)]^2}{n - 2}} \quad (4)$$

$$\Rightarrow S = 0.00279 \text{ mg/l}$$

$$S_{xx} = \sum_{j=1}^n (c_j - \bar{c})^2 = 4.38 \text{ mg/L} \quad (5)$$

$$u(c_0) = \frac{0.00279}{0.12347} \sqrt{\frac{1}{3} + \frac{1}{18} + \frac{(1.53 - 1.75)^2}{4.38}}$$

$$\Rightarrow u(c_0) = 0.0143 \text{ mg/l}$$

Where:

$A_j$  –  $i^{\text{th}}$  measurement of absorbance,

$B_0$  – intercept,

$B_1$  – slope,

$p$  – number of measurements to determine  $c_0$ ,

$n$  – number of measurements for the calibration,

$c_0$  – determined iron concentration of the aliquot solution,

$\bar{c}$  – mean value of the different calibration standards ( $n$  number of measurements),

$i$  – index for the number of calibration standards,

$j$  – index for the number of measurements to obtain the calibration curve.

*Repeatability, rep*

The repeatability was determined using ten repeated measurements of the organo-mineral fertilizer sample which lead to an average of 0.0395% Fe and a standard deviation of 0.0018%. The value of the relative standard deviation,  $RSD = 4.67\%$  can be used directly for the calculation of the combined standard uncertainty.

*Calculating the combined standard uncertainty*

All the intermediate values of the measurement and their standard uncertainties are presented in Table 3.

Table 3. Intermediate values and uncertainties for determination of iron from organo-mineral fertilizers

Description	Value	Standard uncertainty $u(x)$	Relative standard uncertainty $u(x)/x$
Mass of the sample, $m_s$	2 g	0.00044 g	0.00022
Stock solution, $c_{stock}$	100 mg/L	0.2437 mg/L	0.00244
Volume of extracted solution, $V_{100}$	100 ml	0.0508 ml	0.00051
Volume, $V_{100}$	100 ml	0.0508 ml	0.00051
Volume, $V_{20}$	20 ml	0.0139 ml	0.00069
Dilution, $d$			0.00086
Concentration of the aliquot solution, $c_0$	1.53 mg/L	0.0143 mg/L	0.00936
Repeatability, rep	1	0.04670	0.04670

In order to calculate the combined standard uncertainty, the standard uncertainties of each component are used as follows (Eq. 6):

$$\frac{u(c_{Fe})}{c_{Fe}} = \sqrt{\left(\frac{u(c_{ms})}{m_s}\right)^2 + \left(\frac{u(Vc_{stock})}{c_{stock}}\right)^2 + \left(\frac{u(V_{100})}{V_{100}}\right)^2 + u(d)^2 + \left(\frac{u(c_0)}{c_0}\right)^2 + u(rep)^2} \quad (6)$$

$$\frac{u(c_{Fe})}{0.0395} = \sqrt{0.00022^2 + 0.00244^2 + 0.00051^2 + 0.00086^2 + 0.00936^2 + 0.04670^2}$$

$$\Rightarrow u(c_{Fe}) = 0.0019\%$$

The expanded uncertainty  $U_{Fe}$  is calculated by multiplying the combined standard uncertainty by a coverage factor  $k = 2$ , which gives a level of confidence of approximately 95% (Eq. 7). Thus, the content of iron in the organo-mineral fertilizer is  $(0.0395 \pm 0.0038)\%$ . Relative expanded uncertainty is 9.62%.

$$U_{Fe} = 2 \times u(c_{Fe}) = 2 \times 0.0019 = 0.0038\% \quad (7)$$

Figure 2 illustrates the contribution of each component to the uncertainty budget. The contribution of the uncertainty of repeatability is by far the largest (77.73%) followed by the concentration of iron in the aliquot solution (15.58%).

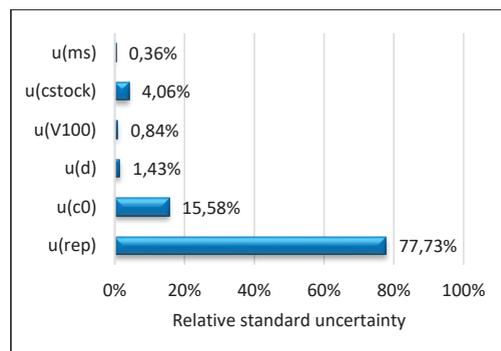


Figure 2. Uncertainty contributions for determination of iron from organo-mineral fertilizers

## CONCLUSIONS

Fertilizer production is a complex and demanding process. As regulatory requirements shift, and awareness of environment issues is growing, the focus on quality control of fertilizers is more intense than ever before. Measuring uncertainty is a key indicator for both fitness-for-purpose of a method and constant reliability of analytical results achieved in a laboratory.

Application of cause-and-effect analysis was used to evaluate the measurement uncertainty

for iron determination in organo-mineral fertilizers by means of atomic absorption spectrometry. The content of iron in the analysed organo-mineral fertilizer sample is  $(0.0395 \pm 0.0038)\%$ . The assessment of various steps of the measurement shows that repeatability, with a contribution of 77.73% was the main component of the uncertainty budget.

## ACKNOWLEDGEMENTS

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## SUBSTANTIATION OF THE PARAMETERS OF THE COMBINE HEADER FEEDER CHAMBER CONVEYOR

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### Abstract

*The materials of the article are devoted to an urgent problem - to reduce losses and crushing of grain by the threshing apparatus of a combine harvester by improving the design and substantiating the operating mode of the conveyor inclined chamber of the header. It describes the design of the proposed chain-slat conveyor for the feeder chamber of the combine harvester, the methodology and results of laboratory studies to substantiate its design parameters and operating mode. Laboratory studies were carried out on a developed experimental setup using the method of planning a multifactor experiment. As an optimization criterion, the degree of uniformity of the distribution of the stalk mass was taken when feeding it into the threshing apparatus of a combine harvester. As a result of laboratory studies, the optimal values of the following parameters were established: the pitch of the stamped strips of the conveyor of the header feeder chamber  $t_p = 307.2$  mm; feeder chamber conveyor speed  $v_{tr} = 3.5$  m/s; working speed of the combine  $v_k = 2.0$  m/s.*

**Key words:** grain harvester, header, inclined body, chain-slat conveyor, grain loss, crushing grain.

### INTRODUCTION

In the complex of works on grain production, the most important and stressful stage is harvesting. For harvesting grain crops, domestic industry and foreign firms produce a wide variety of grain harvesters. The technological process of the reaping parts of modern combine harvesters is not stable, which causes uneven supply of the stalk to the threshing apparatus, which in turn leads to frequent disruptions in the technological process (clogging, jamming of working bodies), a decrease in productivity, a deterioration in the quality of performance, an increase in energy consumption by reaping drive and threshing. The overall reliability of the combine is decreasing (Gubsky, 2018).

An important unit of the reaping part of the combine harvester, which ensures the spreading of the stalk mass coming from the spacer or the finger mechanism of the auger, and its uniform feeding into the threshing apparatus is the floating feeder chamber conveyor.

### MATERIALS AND METHODS

It was found that with direct combining, the initial state of the stem mass entering the feeder

chamber has a minimum in thickness in the middle of the stream and a maximum at the edges (Baizakova, 2012; Baizakova, 2013). To increase the coefficient of uniformity of the supply of the stem mass (increase the degree of leveling), leading to minimization of losses and grain crushing, we have proposed an improved design of the floating feeder conveyor.

The floating conveyor consists of a driving and driven shafts and a chain-slat conveyor. The chain-slat conveyor consists of four parallel sleeve-roller chains 1 (Figure 1) with a pitch of 38.4 mm, to which the fastening plates 3 are connected with rivets 3. Stamped metal strips are fixed to the plates 3 with the help of bolted connections 4 in a staggered manner 2. The length of the strips is 540 mm. The distance between the two middle chains is 443 mm, and the distance between the outermost and the nearest middle chain is 386 mm. The overhang of the outer end of the stamped metal strip relative to the middle of the extreme chain is 105 mm. At the outer ends of the two stamped strips of each extreme row, pins 5 are fixed, designed to exclude cases of clogging of the sidewalls of the inclined body with stem mass. The total web width of the chain-slat conveyor is 1420 mm, and the length is 3379.2 mm.

The stamped strips 4 are fixed to the bushing-roller chains in three rows in a staggered manner. The distance between the planks of one row (pitch) is 307.2 mm, and between adjacent planks of the middle and outer rows -

153.6 mm. This arrangement of the strips allows to significantly improve the uniformity of the supply of the stem mass into the threshing apparatus of the combine (Kukhmazov, 2019).

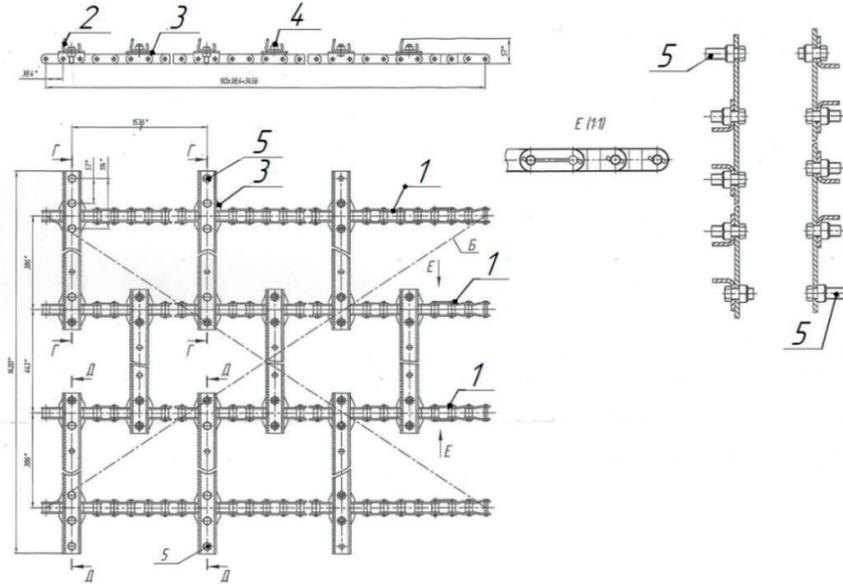


Figure 1. Chain-slat conveyor: 1 - conveyor chain; 2 - stamped bar; 3 - mounting plate; 4 - bolted connection; 5 - pin

To determine the optimal parameters and operating mode of the floating conveyor of the header incline chamber, a three-factor

experiment was carried out on an experimental laboratory setup shown in Figure 2 (Gubsky, 2019).

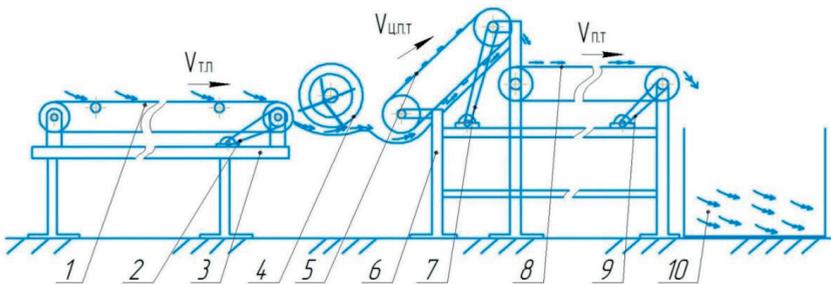


Figure 2. Laboratory setup: 1 - feed conveyor; 2 - feed conveyor drive mechanism; 3 - feed conveyor frame; 4 - auger with a finger mechanism; 5 - chain-slat conveyor; 6 - feeder chamber frame; 7 - drive mechanism; 8 - receiving chamber; 9 - drive mechanism of the receiving conveyor; 10 - collection box

On the basis of a priori information, as well as on the basis of the research tasks, the most significant factors were identified that affect the uniformity of the supply of the stem mass

to the threshing apparatus of a combine harvester. These factors and the levels of their variation are presented in Table 1.

Table 1 - Adjustable factors and levels of their variation

Adjustable factors: natural (coded)	Variation levels		
	-one	0	+1
X1 - speed of the chain-slat conveyor (v <sub>cpt</sub> )	2	3.5	five
X2 - distance between strips (step) (t, mm)	153.6	307.2	460.8
X3 - speed of the feeding conveyor (v <sub>t.p.</sub> , m / s)	1.1	1.8	2.5

The degree of uniformity of the distribution of the stem mass was taken as an optimization criterion.

When determining the coefficient of distribution of the stem mass on the feeding conveyor 1 (Figure 2), a uniform roll 1 m long and 1.41 m wide was formed. The roll weight was 3.3 kg (in accordance with the results of previous studies). The roll of the stalk mass by the feeding conveyor 1 and the finger mechanism of the screw 4 was fed into the inclined chamber, then the chain-slat conveyor directed the roll to the receiving conveyor 8, where measurements were made. The stalk roll was divided into five equal portions along the width of the receiving conveyor (width of each portion 0.28 m) and weighed. Counting the average values of deviations of the masses made it possible to estimate the coefficient of uniformity of the distribution of the stem mass according to the formula (1):

$$v = \frac{m_{max} - m_{min}}{m_{max}}, \quad (1)$$

where:  $m_{max}$  is the maximum portion weight, kg;  $m_{min}$  is the minimum portion weight, kg.

## RESULTS AND DISCUSSIONS

After processing the results of the three-factor experiment in the program "Statistica-6.0"

obtained a mathematical model of the second order describing the dependence of the coefficient of uniformity of the distribution of the crop mass along the width of the receiving conveyor (width of the feeder chamber) on the speed of the chain-slat conveyor  $v_{ts.p.t.}$ , the step of the slats of the chain-slat conveyor  $t_p$  and the feed speed (the speed of the feeding conveyor etc.) in encoded form.

$$v = 98,68444 - 2,44000 \cdot x_1 + 1,52000 \cdot x_2 + 0,62000 \cdot x_3 - 155556 \cdot x_1^2 - 1,05556 \cdot x_2^2 - 1,65556 \cdot x_3^2 \quad (2)$$

The multiple correlation coefficient will be  $R = 0.95$ , the final remainder will be 5.22, a F-test = 0.96. Consequently, the resulting model adequately describes the results of the experiments.

To describe the response surface, a system of differential equations was compiled, which are the partial derivatives of equation 2 for each of the factors. Solving the differential equations, the coded values of the coordinates of the center of the response surface were found and two-dimensional sections were constructed, characterizing the dependence of the coefficient of uniformity of the distribution of the stem mass along the width on significant factors (Figures 3-5).

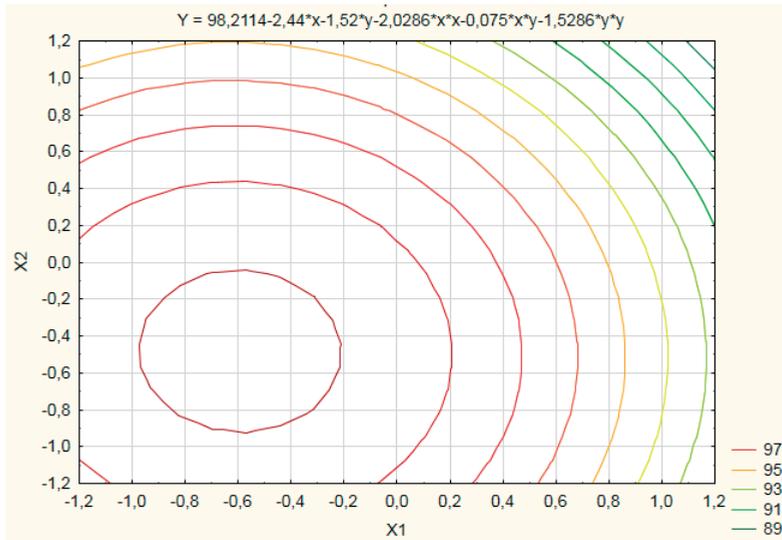


Figure 3. Two-dimensional cross-section of the response surface, characterizing the dependence of the coefficient of uniformity of the distribution of the stem mass along the width on the speed of the chain-plate conveyor  $V_{ts}$ , p.tr. m/s, and the distance between the stamped strips  $t$ , mm

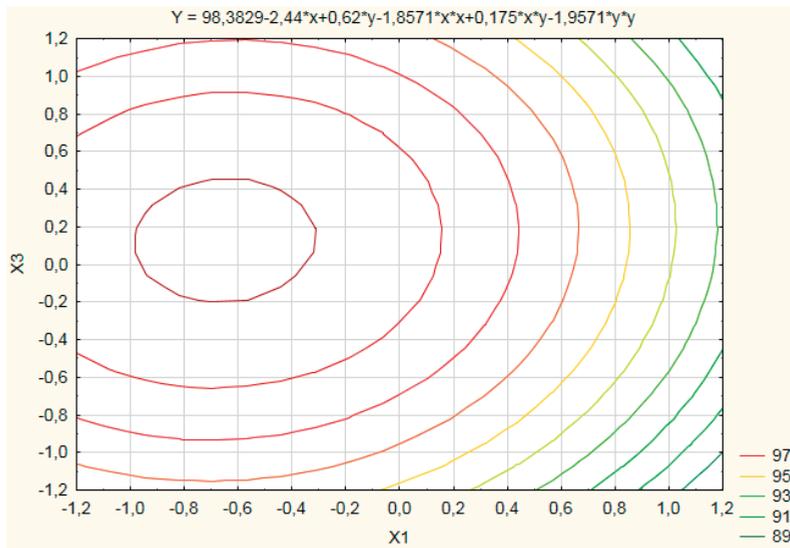


Figure 4. Two-dimensional section of the response surface, characterizing the dependence of the coefficient of uniformity of the distribution of the stem mass over width of the speed of the chain-plate conveyor  $V_{ts}$ , p.tr. m/s, and the speed of the feeding conveyor  $V_p$ , tr., m/s

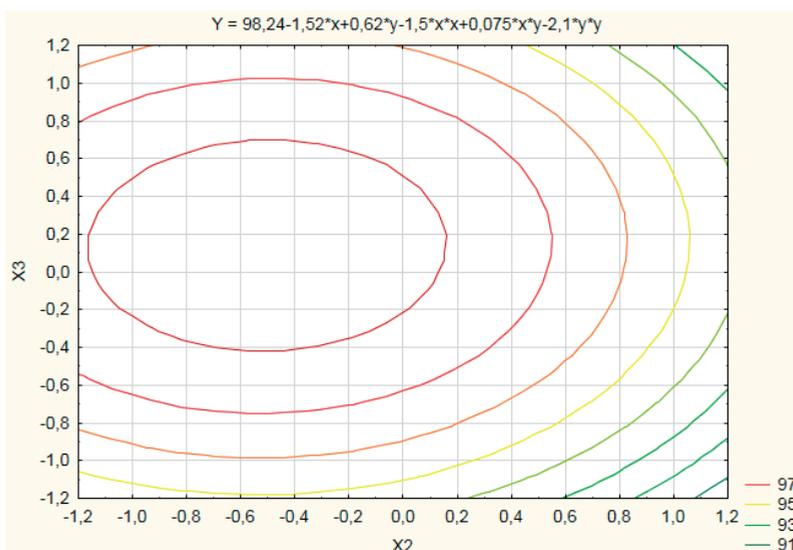


Figure 5. Two-dimensional cross-section of the response surface, characterizing the dependence of the coefficient of uniformity of the distribution of the stem mass along the width on the distance between the stamped strips  $t$ , mm and the speed of the feeding conveyor  $V_{p.tr.}$ , m/s

## CONCLUSIONS

Analyzing the graphic images of two-dimensional sections, it can be concluded that the optimal values of the factors under study are: the speed of the chain-slat conveyor  $v_{ts.p.tr} = 3.5$  m/s; pitch of stamped strip  $p = 307.2$  mm; combine working speed (feed conveyor speed)  $v_{p.tr.} = 2.0$  m/s.

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## THE ESSENTIAL ROLE OF NITROGEN FERTILIZATION UPON PROTEIN AND OIL CONTENT OF MAIZE AND SUNFLOWER YIELDS IN BANAT PLAIN, WESTERN ROMANIA

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### Abstract

*The cereals as maize and oil crops as sunflower are characterized from the point of view of quality parameters by their content in protein and oil. This two important quality parameter are related by the nitrogen fertilization level, the genotype cultivated and environmental, pedological and meteorological conditions during the vegetation period. The experimental fields were located in the Banat Plain, Western Romania, on two different soil types. In the experiment were applied solid nitrogen fertilizers based on urea in different amounts. The experiment results emphasize that three fertilizers UREA-NP, UREA and UREA-ZL were the most efficient ones in case of our studied crops, maize and sunflower.*

**Key words:** *nitrogen fertilizers, protein content, oil content, maize, sunflower.*

### INTRODUCTION

Seed protein and oil content of cereals and oil crops are known to be different from one genotype to the other. The amount of those two parameters is strictly influenced by the level of fertilization, the climate and the fertility of the soil.

In our days, the three leading oilseed crops are soybean, rapeseed and sunflower, the last one being recognized as a major source of high quality edible oil importantly used for culinary purposes. Sunflower contribute almost to 87% of vegetable oil production worldwide, being preferred over other oilseed crops.

Crude sunflower oil has many benefits over the human health, such as: a source of natural antioxidants, anti-inflammatory effects, antidiabetic, antimicrobial and antihypertensive (Adeleke et al., 2020).

The expansion of sunflower crop worldwide is due to a constant evaluation of new cultivars obtained through identification of superior materials capable of expressing high yield and acceptable quality parameters.

Sunflower crops has a great capacity for adaptation and it has interesting characteristics

such as: higher drought resistance than maize, high disease and insect resistance and can improve soil conditions for following crops (Rosa et al., 2009).

One of the most important quality indicator of the sunflower seeds is protein content. The protein content depends on the genotype that is cultivated and the agro-ecological conditions (Balalic et al., 2016; Șmuleac et al., 2020; Ilie et al., 2012).

After FAO, oil type sunflower seeds contain about 38-50% oil and 20% protein, fact that was confirmed also by other authors as Guo et al. (2017), who specify an oil content ranged from 35% to 42%.

After Rosa et al. (2009), the content in protein of sunflower seeds is ranged between 14.73% and 18.23% and oil content may varies from 36.14% to 44.08%.

Maize is a globally important cereal with a high production and productivity, being cultivated in more than 160 countries worldwide, under different agro-climatic conditions. Crop rotation and nitrogen application are among the management methods that can increase maize grain yields. From the nutritional point of view,

maize may contain 7-13% protein and 2-6% oil (Chaudhary et al., 2014).

Nitrogen fertilization strategies for maize is variable, depending on the farmer strategy, but greater than 50% of the dose is applied before planting. Nitrogen losses from denitrification, leaching, volatilization and surface run-off increase with increasing nitrogen availability at planting. A greater synchrony between plant nitrogen demand and nitrogen fertilizer supply can reduce nitrogen losses and improve nitrogen recovery and nitrogen internal efficiencies at a crop level (Fernandez et al., 2020).

Being influenced by the genetic of the hybrid, after the researches made by Marta et al. (2017) in Indonesia, the protein content in maize grain varies between 8.83% and 11.84% and oil content from 4.45% to 7.17%, but most likely, maize seeds may contain 3-5% oil (Ogori, 2020).

Some researchers concluded that after foliar fertilisation the sunflower oil content is increasing from 47.6% to 49.3%.

## MATERIALS AND METHODS

The areas chosen for the establishment of the experimental fields were not random because we followed the effect of the fertilizers tested in different climatic and edaphic conditions.

Testing of solid nitrogen fertilizers was performed in three experimental fields, located in the Banat Plain, Western Romania, during 2019-2020.

Gataia Plain is characterized as a transition zone from the flat plain to the hilly area, located in the southern part of Timiș County, being characterized by the presence of soils classified in the third and fourth quality class, as predominant soil types being: Haplic Luvisol, Haplic Vertisol, Haplic Fluvisol and Haplic Gleysol.

The Mureș Plain which includes the soils belonging to the Cenad locality, is characterized as a low plain, located in the western part of Timiș County. As predominant soil types are found: Haplic Luvisol, Haplic Vertisol, Haplic Fluvisol and Eutric Cambisols. Their fertility is strongly influenced by the presence of the Mureș River, by the presence of

a high clay content and by the seasonal variations of the groundwater level.

The type of soil on which the experiments were placed are Haplic Luvisol in Gataia and Haplic Fluvisol in Cenad (Niță et al., 2018; David et al., 2018).

The solid nitrogen fertilizers used in the experiment were of the urea type, in different concentrations, and with one or two nitrification inhibitors or without one (Tables 1 and 2).

Fertilizers were distributed to both crops in the 6-8 leaf vegetation phase.

From solid nitrogen fertilizers, the same dose were distributed regardless of the type of fertilizer used, for the same crop plant and in all experimental fields.

Table 1. Fertilization variants for sunflower

UREA-Z	350 kg/ha N16:P16:K16 (sowing period)	20 kg	During the vegetation period on each 2000 m <sup>2</sup> experimental plot
UREA-ZL		20 kg	
UREA-NP		20 kg	
UREA-NDP		20 kg	
UREA		20 kg	

Table 2. Fertilization variants for maize

UREA-Z	300 kg/ha 20:20:0+0.05% Zn (sowing period)	30 kg	During the vegetation period on each 2000 m <sup>2</sup> experimental plot
UREA-ZL		30 kg	
UREA-NP		30 kg	
UREA-NDP		30 kg	
UREA		30 kg	

Samples of the plant (seeds) were taken from the harvesting of crops, having a moisture content of 11% and all the necessary conditioning stages were completed before performing the analyses.

Protein content of the seeds was calculated on dependence of the total nitrogen, which was determinate after SR ISO 1871:2002 method. The oil content of the samples was established using SR EN ISO 659.

The statistical analysis used to comment the experimental results of this study, consists in applying the t-Test to compare two averages. This is a null hypothesis test in which the significance limit taken into account is  $\alpha = 0.05$ . As is well known, the existence of significant differences between averages, at the accepted limit  $\alpha$ , is ensured by obtaining a calculated value lower than 0.05. Otherwise, with the same statistical certainty, we will decide that, there are no significant differences between the averages values.

## RESULTS AND DISCUSSIONS

Testing of solid and foliar fertilizers in sunflower and maize crops led to the elaboration of some conclusions regarding their effect on crop quality indicators.

In 2019 was identified a higher protein content in Cenad experimental field, but in 2020 the obtained research data are appropriate for sunflower crop.

The results after the t-Test: Two-Sample Assuming Equal Variances, indicate that we found significant differences between the averages for  $\alpha = 0.05$ , in the experimental field from Cenad, where it was cultivated sunflower, as it is presented in Table 3.

Table 3. The results of the protein content in sunflower seeds (experimental field Cenad)

	<i>Cenad, 2019</i>	<i>Cenad, 2020</i>
Mean	21.082	17.542
Variance	2.39027	3.17207
Observations	5	5
Pooled Variance	2.78117	
Hypothesized Mean Difference		0
Df		8
t Stat	3.35629015	
P(T<=t) one-tail	0.0049933	
t Critical one-tail	1.85954804	
P(T<=t) two-tail	0.00998661	
t Critical two-tail	2.30600414	

In the experimental field data from Gataia, we observed the same significant differences between the fertilization variants, as they were in Cenad, applying the statistical method, which conclude us to the hypothesis that the fertilizers behave in the same regardless the soil type and climatic conditions, in case of sunflower crop (Table 4).

Table 4. The results of the protein content in sunflower seeds (experimental field Gataia)

	<i>Gataia, 2019</i>	<i>Gataia, 2020</i>
Mean	20.066	17.416
Variance	0.15153	1.61513
Observations	5	5
Pooled Variance	0.88333	
Hypothesized Mean Difference		0
Df		8
t Stat	4.45814704	
P(T<=t) one-tail	0.00105813	
t Critical one-tail	1.85954804	
P(T<=t) two-tail	0.00211627	
t Critical two-tail	2.30600414	

The results after the t-Test: Two-Sample Assuming Equal Variances, emphasize significant differences between the averages for  $\alpha = 0.05$ , in Cenad experimental field, for the maize crop, as can be observed in Table 5.

Table 5. The results of the protein content in maize seeds (experimental field Cenad)

	<i>Cenad, 2019</i>	<i>Cenad, 2020</i>
Mean	8.41	7.38
Variance	0.0837	0.7686
Observations	5	5
Pooled Variance	0.42615	
Hypothesized Mean Difference		0
Df		8
t Stat	2.49474398	
P(T<=t) one-tail	0.01862292	
t Critical one-tail	1.85954804	
P(T<=t) two-tail	0.03724585	
t Critical two-tail	2.30600414	

Regarding the protein content of maize seeds, that it can be observed in Tables 5 and 6, highlights amounts almost identical in the both experimental years.

Table 6. The results of the protein content in maize seeds (experimental field Gataia)

	<i>Gataia, 2019</i>	<i>Gataia, 2020</i>
Mean	8.242	7.726
Variance	0.03082	0.68818
Observations	5	5
Pooled Variance	0.3595	
Hypothesized Mean Difference		0
Df		8
t Stat	1.36072467	
P(T<=t) one-tail	0.105347359	
t Critical one-tail	1.859548038	
P(T<=t) two-tail	0.210694718	
t Critical two-tail	2.306004135	

The data collected from Gataia experimental field, in case of maize does not emphasize significant differences, as we observed in Cenad. This result can be explained in the following way: even if the same fertilizers were applied in the same amounts, the delayed period of sowing caused by the climatic conditions lead to this experimental results.

After the t-Test results that we obtained by comparing the oil content of sunflower seeds, we can specify that the differences between the averages are not significant, which mean we obtained almost the same amounts of oil regardless the dose and the type of nitrogen fertilizer (Table 7).

Table 7. The results of oil content in sunflower seeds (experimental field Cenad)

	Cenad, 2019	Cenad, 2020
Mean	28.446	28.066
Variance	10.64583	1.15553
Observations	5	5
Pooled Variance	5.90068	
Hypothesized Mean Difference	0	
df	8	
t Stat	0.247345	
P(T<=t) one-tail	0.405436	
t Critical one-tail	1.859548	
P(T<=t) two-tail	0.810871	
t Critical two-tail	2.306004	

As for the oil content of sunflower seeds, the conditions from Gataia experimental field were more conducive in 2019, but in the following year we observed an unchangeable value of the analysis data (Table 8).

Table 8. The results of oil content in sunflower seeds (experimental field Gataia)

	Gataia, 2019	Gataia, 2020
Mean	30.718	28.066
Variance	9.03137	1.15553
Observations	5	5
Pooled Variance	5.09345	
Hypothesized Mean Difference	0	
df	8	
t Stat	1.8579649	
P(T<=t) one-tail	0.0501217	
t Critical one-tail	1.859548	
P(T<=t) two-tail	0.1002434	
t Critical two-tail	2.3060041	

In case of sunflower cultivated in Gataia, it was found a more favourable response to nitrogen fertilization, regarding the oil content, as in case of Cenad. This fact can be explained that the year 2019 was more favourable for sunflower crop as 2020. Also the climate conditions during the vegetation period of sunflower were more favourable to plant requirements in this period.

## CONCLUSIONS

After interpreting the results obtained from the study, we can formulate the following conclusions: the most effective fertilizer for sunflower in Cenad was UREA-NP and for maize it was UREA.

In the experimental field from Gataia the fertilizer with the best results for sunflower was UREA-NP and for maize UREA-ZL.

We can also conclude that the observed differences are also due to the different pedoclimatic conditions in the two experimental areas, even if the same culture technology and the same fertilization doses were applied.

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## CHANGES OF SOME AGROPHYSICAL PROPERTIES OF AZERBAIJAN DRY SUBTROPICS SOILS USING VARIOUS FERTILIZER SYSTEMS

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### Abstract

*In the irrigated soils of Azerbaijan dry subtropics, the positive effect of the organic fertilizer system on increase in soil aggregates of 10-0.25 mm in size has been established. With the introduction of 40 t/ha of cattle manure, the content of agronomically valuable aggregates in the arable and subarable layers of soils increased on average of 13.0% compared with the control variant. When studying permeability of alluvial meadow-forest zones under apple orchards, irrigated meadow-brown soils under vegetable crops and alfalfa, and also irrigated gray-brown soils under pastures found that the average water absorption rate is, respectively, the above then honed soils -0.0282 m/hour; 0.0691 m/h; 0.0768 m/h and - 0.5664 m/h. For the sustainable and effective functioning of the soil ecosystem, both unnecessarily high and low water permeability undesirable.*

**Key words:** aggregate composition of soils, fertilizer system, water permeability, dry subtropics of Azerbaijan.

### INTRODUCTION

Structural soils are distinguished by favorable physical properties and good nutrients regime, water and air easily penetrate into them. Due to good water permeability, all water falling into the surface of the structural soil is absorbed and not lost. Such soils can provide a solid supply of water in the soil, amounting to 80-85% of the annual rainfall (Kachinsky, 1920, 1963; Guliyev, 2014; Mamedov et al., 2012; Practicum..., 1986).

Numerous studies indicate that the soil structure changes the pace and direction of physicochemical and biological processes in it, affects the plant growth nature and development and crop quality (Karpachevsky, 2005; Mamedov, 1988; Methodology..., 1985).

Changes in soil structure under the influence of mineral and organic fertilizers are not well understood. Analyses of the structural-aggregate composition according to the Savinov method, nitrate nitrogen by the disulfophenolic method, gross forms of nitrogen according to Ginzburg K.E. give very uncertain results, which to a large extent depend on the cultivated crop, tillage, fertilizer system.

Currently, it is relevant to search for ways and methods to restore the ecological functions of

the soil, improve its physical condition and structural-aggregate composition, which lead to the formation of favorable soil properties as a habitat. Optimization of the soil physical state, namely the improvement of its structural-aggregate composition, the preservation of the granular structure, is paramount.

And also when using soils for agricultural purposes, it can lead to a deterioration in their water-permeable properties, which is explained by a change in the structural state, density, porosity, ratio of solid and gas phases due to swelling of hydrophilic soil colloids, etc. The water permeability of the soil, due mainly to its granulometric composition, structural state, density and porosity, is an important condition that affects the leaching of soluble substances, the movement and deposition of food, weathering and soil formation. The nature of the absorption and accumulation of water, the formation of the water balance, depend on it (Agrochemical methods..., 1975; Arinushkina, 1970; Babayev, 1975; Belyuchenko et al., 2013; Dobrovolsky, 1989).

For the sustainable and effective functioning of the soil ecosystem, both excessively high and low water permeability undesirable (Gummatov et al., 1991). With excessive and failed water permeability, the soil has a low

water retention and high evaporation capacity, while low creates the risk of formation surface runoff contributing to the development of water erosion (Gorbyleva et al., 2002; Khasanova et al., 2013; Puzanov et al., 2014; Shane, 2005). Therefore, improving water permeability (including using various doses of organic fertilizers), as well as optimizing the water-physical properties of soils, is one of the most important tasks in the field of soil science, agrochemistry and ecology.

The aim of the research is to establish the influence of different fertilizer systems on the aggregate composition and to identify the water permeability of alluvial meadow-forest irrigated meadow-brown and gray-brown soils under various agrocenoses in the conditions of the dry subtropics of Azerbaijan.

## MATERIALS AND METHODS

The studies were carried out according to the generally accepted methods under various agrocenoses: in the apple orchard on irrigated meadow-brown soils and under vegetable crops (tomatoes) on alluvial meadow-forest soils of the Guba-Khachmaz zone and gray-brown soil under the pastures of Azerbaijan. Agrotechnical measures were consistent with generally accepted agricultural regulations for the region (Lalomova, 2003; Zubkova, 1998).

The agrochemical, physico-chemical and agrophysical properties, as well as the structurally aggregate composition of the studied soils, were studied according to generally accepted methods (Armor, 1985; Barber et al., 2001; Gedroits, 1925).

The structurally-aggregate composition of soils was determined by the method of N.I. Savvinov, density of the addition of the soils solid phase (specific gravity) soil was determined by the psychometric method according to S.N. Dolgov; total porosity - by calculation method; grading - pipette method using a dispersant - sodium pyrophosphate; hygroscopic moisture - by gravimetric method; carbonate ( $\text{CaCO}_3$ ) - by a Scheibler method, absorbed calcium and magnesium - "Trilon B" (displacement of 3N NaCl according to the method of Ivanov; absorbed sodium - according to K.K. Gedroits; total forms of phosphorus and potassium were determined in

apparatus "ContraAA-700" "AnalyticYena" by atomic absorption method; total nitrogen Ginsburg K.E; ammonia nitrogen ( $\text{N-NH}_4$ ), nitrate nitrogen ( $\text{N-NO}_3$ ), mobile phosphorus  $\text{P}_2\text{O}_5$  and exchange potassium  $\text{K}_2\text{O}$  by the colorimetric method (Biernbaum et al., 2005; Gummatov, 2012; Savinov, 1931; Vershinin, 1958). The resulting materials were subjected to mathematical processing on the method (Vershinin, 1958).

Cattle manure was used as organic fertilizer at a humidity of 65%, containing on average 0.5% of nitrogen, 0.25% -  $\text{P}_2\text{O}_5$  and 0.55% -  $\text{K}_2\text{O}$ .

The total area of the experimental plot on irrigated meadow-brown soils was 9600 m<sup>2</sup>, the nutrition area of one tree was 8 x 4 m (32 m<sup>2</sup>). The repetition of experiment was three times. In each variant, the plot area is 1600 m<sup>2</sup>, the total number of trees (together with the second variant) is 48 PCs, of which 9 - accounting (area - 288 m<sup>2</sup>).

Field experiments on alluvial meadow-forest soils under vegetable crops (tomatoes) were laid in triplicate. The plot area was 30 m<sup>2</sup>, the nutritional area of one plant was 2.1 m<sup>2</sup> (70 x 30 cm), in each variant one row is protective:

1. When transferring seedlings to the field;
2. At the beginning of budding;
3. At the beginning of fruiting;

And in fruit orchards under apple trees, mineral fertilizers are introduced:

1. When transferring seedlings to the field;
2. At the beginning of budding;
3. At the beginning of fruiting;

And in fruit orchards under apple trees, mineral fertilizers are introduced:

1. During the period of shoot swelling.
2. After flowering.
3. When tying fruit.

In field experiments, the following options were investigated using organic and mineral fertilizer systems (Table 1).

1. Control (without fertilizer).
2. Cattle manure - 40 t/ha, Organic fertilizer system.
3.  $\text{N}_{60}\text{P}_{60}\text{K}_{120}$  + cattle manure - 20 t/ha, Organic and mineral system.
4.  $\text{N}_{90}\text{P}_{120}\text{K}_{140}$  + cattle manure - 10 t/ha, Organic and mineral fertilizer system.
5.  $\text{N}_{120}\text{P}_{160}\text{K}_{180}$ , Mineral fertilizer system.

Table 1. The application of mineral fertilizers according to the development of plant stages (according to the annual norms of the active substance)

Mineral fertilizers	The periods under the culture of tomato			Periods under the apple tree		
	1	2	3	1	2	3
Nitrogen (%)	30	50	20	20	40	30
Phosphoric (%)	60	30	10	70	20	10
Potash (%)	30	40	30	40	40	20

The water permeability of the soil was determined by the method of filling the sites. For this, metal frames of two sizes were used: internal (accounting) 30 x 30 cm and external (protective) 50 x 50 cm, which prevents water seeping from the registration area and spreading it to the side. Frames deepened into the soil by 15 cm. The area of the water mirror in the registration frame was 0.09 m<sup>2</sup>. In the middle of the reference square, a millimeter scale rail was installed to monitor maintaining a constant water level. Water filling of both sites was carried out simultaneously. Water accounting was recorded at the beginning of the experiment, at the end of the fifth minute, and later, depending on the water permeability of the soil, at 5, 10 and 20 minute intervals. The height of the water layer “h” at the site when determining the absorption rate was 5 cm.

Observations of water absorption by the soil were carried out until a constant absorption rate was established. The resulting materials were subjected to mathematical processing by the method (Gummatov et al., 1991).

All annual norms of organic fertilizers (100%) were plowed in the fall, and mineral fertilizers were applied in 3 terms during the growing season under tomato plants: When filling the site, the level (h) of water was established during the first 10-20 seconds. The amount of water to be poured was measured with 10 liter cylinders. The time report was made by a stopwatch. The experiment continued until the absorption rate was established.

After completion of the field experiment, based on the obtained experimental data, the average rate of water absorption into the soil was calculated. The calculations were carried out according to the method (formulas) of A.N. Kostyakova. The average value of the absorption rate over a period of time  $t$  is determined by the following formula here:

$$K_{av} = \frac{K_0}{t_\alpha}$$

Where:  $K_{av}$  – an average rate of the water absorption mm/min;

$K_0$  – the water absorption rate in the given soil, during the first unit time mm/min;

$$K_0 = \frac{K_1}{1-\alpha}$$

Where:  $K_1$  the rate of water absorption into the soil at the end of the first unit of time, mm/min;

$$K_1 = k_\alpha \cdot t_2$$

Where: the steady rate of water absorption into the soil, mm/min;

$k_\alpha$  - time of completion of infiltration, min;

$t_2$  - time of completion of infiltration, min;

$\alpha$  - the curvature coefficient, the curve characterizing the rate of absorption of water into the soil, is determined by the following formula:

$$\alpha = \frac{\lg k_\alpha t_2 - \lg k_\alpha t_1}{\lg t_2 - \lg t_1}$$

Where:  $t_1$  - time interval accepted during the experiment at the period of infiltration in minutes

## RESULTS AND DISCUSSIONS

The aggregate totality of various sizes, forms of porosity, mechanical strength and water resistance, characteristic for each soil and its horizons, makes up the soil structure, which has long been established by agricultural practices. This is generally recognized, since many soil properties, especially physical conditions, water, air, biological and nutrient regimes, and, consequently, the living conditions of higher plants and microflora, depend on the nature of the soil structure, which is as if focus, where all or at least most of its properties are reflected, the structure to a large extent determines all these properties (Arinushkina, 1970; Mamedov, 2007, 1988; Methodology..., 1985; Mortar et al., 1995; Practicum..., 1986, Skuratov N.S. et al., 2000). The soils of the dry subtropics of Azerbaijan within the Guba-Khachmaz zone are mainly used for agricultural crops. Alluvial meadow-forest soils are poorly provided with basic nutrients. So, in a layer of 0-115 cm of these soils, the humus content is 0.41-3.95%, and the gross forms of nitrogen, phosphorus and potassium vary, respectively, in the range 0.01-0.24%, 0.03-0.16 % and 1.43-3.66% (Table 2).

Table 2. Agrochemical properties of alluvial meadow-forest soil of dry subtropics of Azerbaijan (Guba-Khachmaz zone)

Genetic horizon	Depth, cm	Total humus, %	N-NH <sub>4</sub>				P <sub>2</sub> O <sub>5</sub>			K <sub>2</sub> O			pH H <sub>2</sub> O
			N gross, %	Soluble water, mg/kg	Absorbed, mg/kg	N-NO <sub>3</sub> , mg/kg	Gross, %	Soluble water, mg/kg	Soluble, mg/kg	Gross, %	Soluble water, mg/kg	Exchange, mg/kg	
A ar	0-18	3.95	0.24	4.95	17.4	8.1	0.1	4.22	23	3.8	25	23	7.5
A <sub>1</sub>	18-37	2.87	0.17	3.52	11.2	5.25	0.12	3.05	21.7	3.18	24.7	208	7.8
B <sub>1</sub>	37-65	1.75	0.11	3.05	9.75	3.10	0.09	2.18	13.1	2.56	21.5	198	8.1
B <sub>2</sub>	65-90	1.06	0.04	2.16	6.84	1.08	0.06	1.48	10.2	1.73	17.8	160	8.0
C	90-115	0.41	0.01	1.12	4.92	0.12	0.03	1.19	7.2	1.43	14.7	139	8.1

At the same time, in irrigated meadow-brown soils, these indicators are as follows: humus - 0.64-3.12%; total nitrogen - 0.06-0.29%; total phosphorus - 0.07-0.26%; total potassium - 1.65-3.07% along the soil profile (0-115 cm), which is higher compared with alluvial meadow-forest soils (Table 3). In general, the studied soils of the dry subtropics of the Guba-Khachmaz zone according to the easily assimilated forms of nitrogen, phosphorus and potassium in the upper horizons are characterized as poorly provided, and in terms of acidity, they are almost weakly alkaline (pH 7.5-7.8 units) - Tables 3 and 4.

Table 3. Agrochemical properties of irrigated meadow-brown soil of the Guba-Khachmaz zone

Genetic horizon	Depth, cm	Total humus, %	N-NH <sub>4</sub>				P <sub>2</sub> O <sub>5</sub>			K <sub>2</sub> O			pH H <sub>2</sub> O
			N gross, %	Soluble water, mg/kg	Absorbed, mg/kg	N-NO <sub>3</sub> , mg/kg	Gross, %	Mobile soluble, mg/kg	Soluble, mg/kg	Gross, %	Soluble water	Exchange, mg/kg	
A ar	0-22	3.12	0.29	6.08	20.1	12.2	0.26	8.07	35.8	3.07	26.2	264	7.8
A <sub>1</sub>	22-43	2.58	0.20	5.14	16.9	11.6	0.19	5.96	27.6	2.54	16.3	187	7.7
B <sub>1</sub>	43-70	2.35	0.12	3.72	15.3	5.07	0.14	4.35	18.3	1.92	4.05	109	7.9
B <sub>2</sub>	70-92	1.26	0.10	3.91	13.9	1.15	0.11	3.72	12.8	1.76	3.12	83.7	8.2
C	92-115	0.64	0.06	2.60	7.35	0.86	0.07	2.63	8.92	1.65	2.23	72.1	8.3

The absorbed Ca<sup>2+</sup> and Na<sup>+</sup> cations prevailed in alluvial meadow forest soils (Ca<sup>2+</sup> - 78.1%; Na<sup>+</sup> - 5.0%). The Mg<sup>2+</sup> content was 16.8%. At the same time, in irrigated meadow-brown soils, compared to alluvial meadow-forest soils, Mg<sup>2+</sup> prevailed in the upper horizons, the content of which in the soil layer of 0-22 cm reached 30.0% of the total absorbed cations. In these horizons, the content of absorbed Ca<sup>2+</sup> was equal to 66.9%, Na<sup>+</sup> - 3.1% of the total cations. The inter-aggregate total porosity in the upper horizons of alluvial meadow-forest soils varied between 37.5-45.7% and varied from 37.2 to

40.3% of the total porosity. In the irrigated meadow-brown soils, the total porosity in the upper horizons was 44.0-43.6%, in the lower horizon C decreased to 39.3%.

Table 4. Physico-chemical and agrophysical properties of alluvial meadow-forest soil of dry subtropics of Azerbaijan (Guba-Khachmaz zone)

Genetic horizon	Depth, cm	CO <sub>2</sub> , %	Absorbed bases mEq/100 g soil					The proportion of absorbed cations, % from the sum			Total porosity, %	Hygroscopic moisture, %	Granulo-metric composition, %	
			CaCO <sub>3</sub> , %	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Na <sup>+</sup>	sum	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Na <sup>+</sup>			<0.01 mm	<0.01 mm
A ar	0-18	1.61	3.66	28.8	6.2	1.86	36.9	78.1	16.8	5.04	45.7	2.95	19.8	38.1
A <sub>1</sub>	18-37	1.82	4.14	31.6	7.4	1.72	40.7	77.6	18.2	4.22	37.5	3.42	16.5	39.1
B <sub>1</sub>	37-65	1.59	3.62	32.1	13.8	3.36	49.3	65.2	28.0	60.8	40.3	3.98	25.0	58.2
B <sub>2</sub>	65-90	1.75	3.98	24.6	20.5	2.85	48.0	51.3	42.8	5.94	39.1	4.75	17.2	32.4
C	90-115	1.48	3.37	18.8	22.7	2.14	43.6	43.1	52.0	4.90	37.8	5.20	12.3	28.7

In close connection with the physical properties of the studied soils their water features. The value of humus and hygroscopic moisture varied according to the profile depending on the content of physical clay: in alluvial meadow-forest soil the humus content was 2.95% in the upper and 5.20% in the lower layers, in the irrigated meadow-brown soil - 4.11% and 3.91%, respectively. The studied alluvial soils are carbonate - and the CaCO<sub>3</sub> content on the soil profile (0-115 cm) varied from 3.37% to 3.66% in meadow-forest and from 7.95% to 11.7% in meadow brown soil (Table 5).

The number of agronomically valuable water-resistant aggregates (<0.25 mm) on profile in alluvial meadow-forest and irrigated meadow-brown soils varied respectively in the range of 27,8-46,2 and 26.6-46.6%. Soil conditions along with soil density and productive moisture reserves also determine such indicators as the structural coefficient and the number of agronomically valuable aggregates. The combination of macro-aggregates of soil particles on various shapes and sizes over 0.25 mm forms aggregate composition of the soil, it can be considered as an object that reflects the results of not only soil-forming processes, but also agricultural activity.

An analysis of the data shows that the use of an organic (cattle manure) fertilizer system improves the structural condition of soils. The use of organic-mineral (manure + nitrogen, phosphorus and potassium fertilizers) fertilizer systems positively influenced the structural

state of alluvial meadow-forest irrigated meadow-brown soils under fruit and vegetable. On average, over three years with the introduction of organic fertilizers (cattle manure 65% moisture) under tomatoes in for alluvial meadow-forest soil, an increase in the fraction of the valuation range (0.25-10.0 mm) in 0-60 cm soil layer was noted, where the structural coefficient (Kst) in comparison with the non-fertilized version increased on average to 0.7 units.

Table 5. Physico-chemical and agrophysical properties of irrigated meadow-brown soil of dry subtropics of Azerbaijan (Guba-Khachmaz zone)

Genetic horizon	Depth, cm	CaCO <sub>3</sub> , %	Absorbed bases mEq/ 100 g soil				For absorbed cations mg/ 100 g soil			Hygroscopic moisture, %	Total porosity, %	Particle size distribution structure, %	
			Ca <sup>2+</sup>	Mg <sup>2+</sup>	Na <sup>+</sup>	sum	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Na <sup>+</sup>			<0.001mm	<0.01mm
A ar	0-22	7.95	28.3	12.7	1.32	42.4	66.9	30.0	3.11	4.08	43.6	20.3	36.8
A1	22-43	8.37	31.0	5.12	1.81	37.9	81.7	13.5	4.77	4.11	44.0	17.5	37.2
B1	43-70	9.05	32.9	7.56	1.28	41.7	78.8	18.1	3.06	3.92	41.6	27.2	54.7
B2	70-92	13.9	25.5	13.9	2.65	42.0	60.7	33.0	6.30	3.28	40.9	14.6	33.5
C	92-115	11.7	24.0	12.6	1.72	38.3	62.7	32.9	4.49	3.70	39.3	11.8	27.3

Preservation of a unique gummy-granular structure and well distinguishable soil lumps and aggregates is an important function of the soil after exposure of water.

The change in the aggregate composition and structural coefficient of alluvial meadow-forest soil under vegetable crops (tomato) depending on the fertilizer system (average data for 2014-2016) are shown in Table 6.

The results of determining the content of structural aggregates showed that when applying organic as well as organic-mineral fertilizers, this property was higher compared to the variable control. The content of soil aggregates with a value less than 0.25 mm in the 0-60 cm soil layer in the control variant averaged 42%, where as in the variant where the system of organic (40 t/ha of manure) and organic-mineral fertilizers (20 t/ha manure + N<sub>60</sub>P<sub>90</sub>K<sub>120</sub>), these values were 31% and 33%, respectively.

Moreover, in these variants, the average content of agronomically valuable aggregates of 10.0-0.25 mm in size increased to 9.2%.

An analysis of the obtained data shows that the use of organic and organic-mineral fertilizer systems on irrigated meadow-brown soil also positively affected the structural state of soils in comparison with the control variant. So, the content of soil aggregates of <0.25 mm in the arable horizon in the control version is 46.2%, while using organic fertilizers (40 t/ha of manure), these indicators decreased to 28.7%, and the content of agronomically valuable particles in the amount of 10.0-0.25 mm increased to 71.3% in alluvial meadow-forest soil under tomato cultivation.

When using the organic-mineral fertilizer system, an increase in the content of particle sizes (10.0-0.25 mm) from 66.2% to 71.3% in the arable layer of soils under vegetable crops was also established.

When using fertilizers at a dose of N<sub>60</sub>P<sub>90</sub>K<sub>120</sub> + 20 t/ha of manure (cattle), the content of soil agronomically valuable aggregates in the arable layer of soils was 63.5%, and in the sub-arable one it reached 67.8%. The structural factors were 1.7 and 2.1, respectively.

When applying only mineral fertilizers in a dose of N<sub>120</sub>P<sub>160</sub>K<sub>180</sub>, a positive effect on the content of soil aggregates 10.0-0.25 mm in size (agronomically value) was not established in comparison with the control variant, but on the contrary, the content of particles <0.25 mm in size increased slightly, that led to a deterioration in water-physical soil indicators.

When using an organic fertilizer system (40 t/ha of cattle manure), the best indicators were found in the content of agronomically valuable particles with a size of 10.0-0.25 mm, which was 60.2-53.7% in a layer of 0-60 cm soil, on average arable the layer was 66.8%. Also, high values of the structural coefficient were noted. In the arable and subsurface layers of the alluvial meadow-forest soil under the tomato culture, these indicators were 1.2 and 1.4, respectively.

If in the control variant the average content of soil aggregates with a particle size of 10.0-0.25 mm in the 0-60 cm soil layer was 60.8% (60.76), then when using organic (40 t/ha of manure) and organic-mineral (20 t/ha of manure + N<sub>60</sub>P<sub>90</sub>K<sub>120</sub>) of fertilizer systems, these figures are respectively equal to an average of 68.2 and 66.8% (Table 6).

An analysis of these data shows that, on average for 3 years of research using an organic-mineral fertilizer system in irrigated meadow-forest soil under tomatoes at the end of the growing season, the content of agronomically valuable aggregates (10.0-0.25 mm) increases to about 9.2% and this is the best result compared with the control option in the experiment.

Table 6. The influence of the fertilizer system on the aggregate composition and structural coefficient of alluvial meadow-forest soil under vegetable crops (tomato) (Guba-Khachmaz zone, 2014–2016)

Fertilizer system	Fertilizer dose	Soil layer, cm	The size of soil aggregates, content, %			Structural coefficient (K <sub>s</sub> )
			>10	10-0.25	<0.25	
Without fertilizer (control)	–	0-20	0.1	53.7	46.2	1.2
		20-40	0.3	59.1	40.6	1.4
		40-60	0.4	60.2	39.4	1.5
Organic fertilizer	40 t/ha	0-20	–	71.3	28.7	2.5
		20-40	–	67.8	32.2	2.1
		40-60	0.1	61.2	38.8	1.6
Organic mineral	N <sub>60</sub> P <sub>90</sub> K <sub>120</sub> + 20 t/ha manure	0-20	0.2	67.8	32.2	2.1
		20-40	0.4	68.9	27.8	2.2
		40-60	–	63.8	36.2	1.8
Organic mineral	N <sub>60</sub> P <sub>90</sub> K <sub>140</sub> + 20 t/ha manure	0-20	0.3	66.2	33.5	2.0
		20-40	0.1	62.5	37.4	1.7
		40-60	–	58.8	41.2	1.4
Mineral	N <sub>120</sub> P <sub>180</sub> K <sub>180</sub>	0-20	0.4	59.4	40.2	1.5
		20-40	0.2	61.1	38.7	1.6
		40-60	0.5	57.2	42.3	1.3
SED <sub>0.05</sub> (the smallest essential difference)						<b>0.18</b>

It was established that with the joint application of mineral and organic fertilizers with an increase in the doses of mineral fertilizers and a decrease in the doses of organic fertilizers, the content of soil aggregates of 10.0-0.25 mm in size gradually decreased, that is, a decrease in the doses of organic fertilizers negative affected the content of agronomically valuable aggregates.

The structural coefficient in the meadow-brown soil in the control (without fertilizers) variant at 0-60 cm layer averaged 1.7; with an organic fertilizer system, the average value was 2.2, and in the version where an organic-mineral fertilizer system (N<sub>60</sub>P<sub>90</sub>K<sub>120</sub> + 20 t/ha of manure) was used, 2.0.

The structural-aggregate composition of soils in the of the organic-mineral system compared to the organic fertilizer system turned out to be lower in the version of the organic system (40 t/ha manure) where the structural coefficient was 2.17, and 0 in the option 20 t/ha manure + N<sub>60</sub>P<sub>90</sub>K<sub>120</sub> this value was 2.0. The average value of K<sub>st</sub> in the layer of 0-60 cm was 6.7.

It was revealed that the use of the mineral fertilizer system reduces the structural coefficient, which is on average 1.4 over the soil profile. In addition, the content of soil

aggregates in the soil with a particle size <0.25 mm increases, that worsens the soil structure and negatively affects the chemical soil environment. When studying the aggregate composition and structural coefficient in an irrigated meadow-brown soil under fruit trees, the similar results were obtained.

According to the experience in the general studied soils under different agrocenoses, the best option was to use an organic fertilizer system. The structural coefficient in the alluvial meadow-forest soil was: 2.1-2.5 in the arable layer, and 2.0-2.4 in the irrigated meadow-brown soil. Changes in the aggregate composition and structural coefficient of irrigated meadow-brown soil under fruit crops (apple tree) depending on the fertilizer system are shown in Table 7.

The water permeability of soils of the dry subtropics under various agrocenoses was also studied. The water permeability of soils was studied according to the above method on alluvial meadow-forest and irrigated meadow-brown soils of the Guba-Khachmaz zone.

Table 7. Aggregate composition and structure coefficient in the irrigative meadow-brown soils under fruit (apple) cultures depending on fertilizer systems (Guba-Khachmaz, 2014-2016)

Fertilizer system	Fertilizer norm	Soil layer, cm	Size of the soil aggregates, mm			Structure coefficient K(st)
			>10	10-0.25	<0.25	
Unfertilized (control)	–	0-20	2.6	52.6	45.4	1.2
		20-40	2.1	57.0	40.9	1.3
		40-60	4.3	72.7	23.0	2.7
Organic	40 t/ha manure	0-20	1.8	66.2	32.0	2.0
		20-40	1.4	70.5	28.1	5.4
		40-60	3.9	67.8	28.3	2.1
Mineral organic (joint application)	N <sub>60</sub> P <sub>90</sub> K <sub>120</sub> + 20 t/ha of manure	0-20	2.0	63.5	34.5	1.7
		20-40	1.7	67.8	30.5	2.1
		40-60	2.2	69.1	28.7	2.2
Mineral organic (joint application)	N <sub>60</sub> P <sub>100</sub> K <sub>140</sub> + 20 t/ha of manure	0-20	1.7	61.8	36.5	1.6
		20-40	1.5	63.7	34.8	1.8
		40-60	2.6	66.8	30.6	2.0
Mineral	N <sub>120</sub> P <sub>180</sub> K <sub>180</sub>	0-20	2.7	50.7	46.6	1.0
		20-40	2.3	54.8	42.9	1.2
		40-60	3.8	69.6	26.6	2.3
SED <sub>0.05</sub> (the smallest essential difference)						<b>0.27</b>

The research results are presented in the following order. In the irrigated meadow-brown soils of the Guba-Khachmaz region in the zone of meadow-forest soils reserved for growing vegetables and alfalfa, the water permeability of soils was studied. The results of field studies under various agrocenoses on soil water permeability are also given in Tables 8 and 9.

Based on the results of field work to determine the water permeability of soils in various experimental plots according to the formulas of A.N. Kostyakov, a calculation was made to determine the average rate of water absorption in the experimental plots.

Table 8. The results of field experiments to determine the water permeability of soils.(Guba-Khachmaz zone)

Experimental plots	The time interval taken during the experiment $t_1$ minutes	Time to complete the infiltration $t_2$ minutes	The rate of absorption of water into the soil at the end of the first unit of time $mm / min$ $K_1$	Steady soil absorption rate $mm / min$ $K_2$
Alluvial meadow-forest soil under vegetable	1	65	2.60	0.15
Irrigated meadow-brown soil under apple orchards crops	1	66	6.51	0.31
Irrigated meadow brown soils under alfalfa culture	1	61	6.20	0.50

The calculation procedure for determining the average rate of soil absorption is given in the "Research Methodology" section. The calculation results are shown in the Table 8.

From Table 9 it is seen that the average rate of absorption of water into the alluvial-meadow-forest soils of the Guba-Khachmaz zone under apple orchards is 0.47 mm/min or 0.0282 m/h. In the plot of irrigated meadow-brown soils of the Guba-Khachmaz zone under vegetable crops, the average rate of absorption of water into the soil is 0.0691 m/h, and under the alfalfa culture, the average rate of absorption of water into the soil is 0.0768 m/hour (Table 9).

Table 9. The results of determining the rate of absorption of water in various types of soil under various agrocenoses (Guba-Khachmaz zone)

Experimental plots	Calculation results				
	$\alpha = \frac{\lg k_2 - \lg k_1}{\lg t_2 - \lg t_1}$	$k_1 = k_2 \cdot t_2^\alpha$	$k_0 = \frac{k_1}{1-\alpha}$	$k_{av} = \frac{k_0}{t_2^\alpha}$	
	-	mm/min	mm/min	mm/min	m/h
Alluvial meadow-forest soil under vegetable crops	0.68	2.56	8.0	0.47	0.0282
Irrigated meadow-brown soil under apple orchards	0.73	6.62	24.53	1.15	0.0691
Irrigated meadow-brown soil under culture alfalfa	0.61	6.20	15.70	1.28	0.0768

Meadow-brown soils are characteristic representatives of a number of hydromorphic soils of the moderate dry subtropics of Azerbaijan. In their geographical confinement, they are locally distributed among the brown soils of the foothill plains of the Great and

Little Caucasus, including the Guba-Khachmaz Plain, where periodically surface and ground moisture are available.

These soils are formed under thinned forests and shrubs with well-developed grass stands. A significant area of meadow-brown soils is plowed and used for orchards and crops.

Soil-forming rocks are diluvial - proluvial and ancient alluvial deposits of clay-loamy composition or small-earth pebble, often carbonate, sediments of mountain river outflow cones. The influence of groundwater and surface runoff on soil formation is periodic. Groundwater lies at a depth of 2.5-5.0 and deeper. In the soils described, biological processes occur with moderate moisture (10-25%) and temperature (18-23°C), the vital activity of microorganisms occurs under normal conditions.

The characteristic morphological features of meadow-brown soils are: the presence of well-defined grayish-brown or dark-humus horizon (AUvz) with a thickness of 30-35 cm, a granular-lumpy structure, noticeable clayness and compaction of the middle part of the profile with the lumpy-nutty structure (Btg- 35-40cm), separation of relatively loose gleying of low horizons (Cgca).

## CONCLUSIONS

An analysis of the agrochemical and agrophysical indicators of the studied alluvial meadow-forest and irrigated meadow-brown soils of the Guba-Khachmaz zone of Azerbaijan showed that the following measures are necessary to increase their fertility: application and improvement of mineral and organic fertilizer systems.

In the variant where the organic fertilizer system was used (40 t/ha of manure), the highest indicators of improvement and conservation of agronomically valuable soil aggregates of alluvial meadow-forest soil were revealed. At the same time, the values of the structural coefficient in the arable and subsurface layers of the soil are 2.5 and 2.1, respectively.

When using the organic-mineral fertilizer system under vegetables in the  $N_{60}P_{90}K_{120} + 20$  t/ha manure variant, the aggregate composition of the studied soils is also improved - the

content of agronomically valuable aggregates in the arable and subsurface layers of the soil increased in comparison with the control (without fertilizers) option, respectively, by 2.1 and 2.2%.

The structural coefficient increased with the organic fertilizer system compared with the control by about 1.3 times.

Alluvial meadow-forest soils of the Guba-Khachmaz zone under apple orchards belong to the group of poorly permeable soils.

The irrigated meadow-brown soils of the Guba-Khachmaz zone under vegetable crops and under alfalfa belong to the medium-permeable soil groups.

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## HEAVY METAL UPTAKE PATTERNS BY SPINACH (*Spinacia oleracea* L.) GROWN ON CONTAMINATED DIFFERENT SOIL TYPES

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### Abstract

*Heavy metals have different behaviour on crop plants, depending on several factors such as metal type, the quantity and mixture of metals in soil and soil type. Both pedological and agrochemical soil characteristics can influence heavy metals mobility and bioavailability, with notable consequences on plant growth. This study follows the accumulation of heavy metals and growth of spinach plants (*Spinacia oleracea* L.) on three different soil types (chernozem, fluvisol, chromic luvisol- acc. WRB-SR, 1998), each soil type having six variants (1 untreated soil + 5 contaminated soils) with different concentrations of heavy metals. The contaminations were made with five mixtures of heavy metals (Cd, Pb, Ni, Co, Cu and Zn) in quantities ranging near reference values to values that exceeds intervention threshold for sensitive soils, according to Ord. 756/1997 (Cd from 3 to 20 ppm, Pb from 30 to 150 ppm, Ni from 25 to 200 ppm, Co from 20 to 100 ppm, Cu from 20 to 200 ppm and Zn from 100 to 700 ppm). Soil and plant analysis for heavy metals determination were made using ICP-MS technique. The experimental results showed a significant difference on growth parameters of spinach plant and on heavy metals bioaccumulation between untreated and high contaminated soils variants, and between soil types, respectively.*

**Key words:** bioaccumulation, heavy metals, soils, *Spinacia oleracea* L.

### INTRODUCTION

Nowadays, soil degradation (e.g., erosion, loss of organic matter, sealing, pollution) is an increasing problem all over the world. Contamination by heavy metals is considered as one of the main threats due to their potential accumulation in bio-systems through contaminated water, soil and air (Pauget et al., 2015). A better knowledge of heavy metal sources, their accumulation in the soil and the effect of their presence on plant system is a fundamental issue for studies on risk assessment (Salawu et al., 2015).

All soils contain very low levels of heavy metals, naturally. The presence of them in soil does not necessarily mean contamination. The concentration of metals in uncontaminated soil is most often related to the geology of the parent material from which the soil was created (McLean & Bledsoe, 1992). Metals found in the aqueous phase of the soil are prone to movement with soil water and can be transported to groundwater by leaching processes. Metals, unlike some toxic organic

compounds, cannot be degraded. But most of them can be transformed by chemical processes into more or less stable compounds that can reduce their toxicity and mobility. The immobilization of metals by adsorption and precipitation processes can prevent both accumulation in plants and leaching in groundwater. Even though they are toxic in large quantities, some heavy metals are essential for the normal growth of plants, and for some of them their biological role is still difficult to establish.

Most heavy metals are hardly soluble, with low mobility. Many times, metals are found in the soil as residual forms, due to strong bonds with organic and inorganic ligands. For example, Pb and Cu are found mostly in complexed form, while Zn and Cd are found in ionic form in the soil solution (Shahid, 2017).

There is a dynamic equilibrium in soil between liquid and solid phases. When the concentration of an element exceeds the equilibrium state, then it interacts with other elements in the soil, changing the normal balance. This balance depends on the nature of the soil, structure,

chemical composition, organic matter, pH and many other characteristics (Butnariu, 2012). Soil reaction (pH) has been described in many studies as one of the main factors influencing the mobility of heavy metals in soil. Hou et al. (2019) found that the available content of Cu, Zn and Pb was negatively correlated with soil pH, while organic matter (OM) content was positively associated with Zn availability. Another study on the influence of pH on heavy metal mobility showed that the Cd, Cu and Zn depends on soil pH and the decrease from the value of 8.1 to 7.2 lead to a larger content of Cd, Cu and Zn (Vrancuta et al., 2019).

The solubility of heavy metal compounds, especially minerals, oxides or carbonates depends on the pH value. In general, the decreasing tendency of the pH leads to release of cations and an increase of the pH leads to release of free anions (Król et al., 2020).

The amount of available soil OM significantly influences metal bioavailability since it is considered one of the most important soil constituents that retains heavy metals (Aigberua, 2018). Organic matter appears to have the greatest capacity for sorption of trace elements in cationic form. Humic substances contain a large number of complexation centers, therefore they behave as a natural multiligand system (Violante et al., 2010).

Many studies report on the interactions between different heavy metals and their competition for binding sites. For some adsorption sites, cationic heavy metals are preferentially adsorbed instead of the major cations (Mg, Ca) and anionic metals are preferentially adsorbed over major anions (SO<sub>4</sub><sup>2-</sup>, NO<sub>3</sub><sup>-</sup>) (McLean & Bledsoe, 1992). Trace metals also will compete with each other for adsorption sites. The presence of other cations, either major elements or heavy metals, can significantly affect the mobility of a particular element. Some studies have looked at interactions between heavy metals, but have generally grouped two or a maximum of three elements. The Cu, Zn and Cd interaction was observed by Kuo & Baker (1980), Ueno et al. (2004), Zemanová et al. (2014) and many more. Other interactions were studied between Ni-Cd (Davari et al., 2014), Zn-Ni-Cu (Hossain et al., 2009) and many more combinations.

This study aims to determine the accumulation of various heavy metals in spinach plants, using increasing concentrations starting from normal soil values, to values that exceed the norms described in the legislation in force. Three types of soils with different characteristics are subjected to the same contamination conditions, in order to observe their influence on the accumulation of heavy metals.

## MATERIALS AND METHODS

### Soil Characterization, Sampling and Processing

Three types of soil from different regions of Romania were selected for this study. The soils were taken from three representative areas for the type of soils, as presented in Table 1.

Table 1. The soils used experiment

Code	Type	Region	Photo
S1	<i>Chernozem</i>	Near Fundulea (Calarasi) GMS 44°29'34.0"N 26°29'47.2"E	
S2	<i>Chromic luvisol</i>	Moara Domneasca (Ilfov) GMS 44°29'57.7"N 26°15'09.7"E	
S3	<i>Fluvisol</i>	Near Belciug (Prahova) GMS 44°48'57.8"N 26°15'26.9"E	

Some of the pedological and agrochemical properties of these soils, at the time of collection, are presented in Table 2.

Table 2. Soil characteristics

Characteristic	S1	S2	S3
pH	6.53	6.45	7.8
EC (μS/cm)	64.53	102.23	200.03
Organic carbon (%)	2.06	1.61	2.48
Organic matter (%)	3.55	2.77	4.27
Total N (%)	0.17	0.16	0.25
Bulk density (g/cm <sup>3</sup> )	1.20	1.39	1.26
Total porosity (%)	48.38	44.37	49.31
Soil compaction (%)	3.23	-12.34	12.56

The soil was collected from the areas indicated in the previous paragraph, from a depth of 0-20 cm (rhizosphere), in polyethylene bags to keep its moisture, until the end of the collecting period. Excessive soil drying has been avoided, to be easily processed for the following steps. After collecting the entire required amount, the soils were cleaned of plant remains, stones and other impurities, crushed by hand and then sifted through a 7x5 mm mesh horticultural sieve. Each type of soil was homogenized and kept in polyethylene bags until the entire quantity was conditioned.

### Greenhouse experiment

For the establishment of the spinach crop, 3-liter pots were used. The vessels were covered in polyethylene bags to prevent the loss of excess water in case of over-irrigation. Loss of water means loss of soluble salts from the soil, leading to a decrease of heavy metals concentration. The pots were placed on plates, numbered, coded and then filled with 3 kg of soil (Figure 1). The vessels were placed in one of the USAMV Bucharest greenhouses.

The biological material was represented by spinach seeds (*Spinacia oleracea* L.), Matador variety. Six soluble salts were used, Cd(NO<sub>3</sub>)<sub>2</sub>•4H<sub>2</sub>O, Pb(CH<sub>3</sub>COO)<sub>2</sub>•3H<sub>2</sub>O, Ni(NO<sub>3</sub>)<sub>2</sub>•6H<sub>2</sub>O, Co(NO<sub>3</sub>)<sub>2</sub>•6H<sub>2</sub>O,

Cu(NO<sub>3</sub>)<sub>2</sub>•3H<sub>2</sub>O, Zn(CH<sub>3</sub>COO)<sub>2</sub>•2H<sub>2</sub>O as soluble form of Cd, Pb, Ni, Co, Cu and Zn.



Figure 1. Preparing experimental pots

Five mixtures were prepared, with different concentrations of each metal, resulting in five variants of treatments, denoted as C1, C2, C3, C4, C5. The concentrations were chosen to cover as much as possible the whole interval, from reference value to values that exceed intervention threshold for sensitive use of soils, as described by Romanian regulation (Order 756/1997). Untreated soils were used as control variants (C0). It must be taken into account that the initial soils had their specific load of heavy metals, to which was added the amounts established in the experiment, resulting in the quantities in Table 3.

Table 3. Concentrations of heavy metals used for soil contamination on each variant

Metal	Initial amount of heavy metals in control (C0) (ppm)	C1 (ppm)	C2 (ppm)	C3 (ppm)	C4 (ppm)	C5 (ppm)
Cd	Vi	Vi+3	Vi+5	Vi+10	Vi+15	Vi+20
Pb	Vi	Vi+30	Vi+50	Vi+75	Vi+100	Vi+150
Ni	Vi	Vi+25	Vi+75	Vi+100	Vi+150	Vi+200
Co	Vi	Vi+20	Vi+30	Vi+50	Vi+75	Vi+100
Cu	Vi	Vi+ 20	Vi+60	Vi+100	Vi+150	Vi+200
Zn	Vi	Vi+100	Vi+300	Vi+450	Vi+600	Vi+700

Five concentrations were used, along with the initial soil used as control, resulting in six treatment variants for each soil type (Table 4). Having three types of soil, six variants of concentrations and four replicates, resulted in a total of 72 pots, respectively 216 kg of conditioned soil.

After preparing the pots, the contaminations were performed with the previously prepared solutions, at the second irrigation, after 24

hours from the first. The first irrigation was carried out using an amount of water of approximately 10% of the amount of soil, 300 ml of water/pot. The amounts of metals calculated for each concentration were dissolved in 300 ml of distilled water and added to each pot. The seeds were planted 48 hours after contamination, after the soil solution was stabilized.

Table 4. Experimental scheme

		Soil type											
		Chernozem (S1)				Chromic luvisol (S2)				Fluvisol (S3)			
Replicates Conc.		Rep 1	Rep 2	Rep 3	Rep 4	Rep 1	Rep 2	Rep 3	Rep 4	Rep 1	Rep 2	Rep 3	Rep 4
	Control C0		S1C0R1	S1C0R2	S1C0R3	S1C0R4	S2C0R1	S2C0R2	S2C0R3	S2C0R4	S3C0R1	S3C0R2	S3C0R3
C1		S1C1R1	S1C1R2	S1C1R3	S1C1R4	S2C1R1	S2C1R2	S2C1R3	S2C1R4	S3C1R1	S3C1R2	S3C1R3	S3C1R4
C2		S1C2R1	S1C2R2	S1C2R3	S1C2R4	S2C2R1	S2C2R2	S2C2R3	S2C2R4	S3C2R1	S3C2R2	S3C2R3	S3C2R4
C3		S1C3R1	S1C3R2	S1C3R3	S1C3R4	S2C3R1	S2C3R2	S2C3R3	S2C3R4	S3C3R1	S3C3R2	S3C3R3	S3C3R4
C4		S1C4R1	S1C4R2	S1C4R3	S1C4R4	S2C4R1	S2C4R2	S2C4R3	S2C4R4	S3C4R1	S3C4R2	S3C4R3	S3C4R4
C5		S1C5R1	S1C5R2	S1C5R3	S1C5R4	S2C5R1	S2C5R2	S2C5R3	S2C5R4	S3C5R1	S3C5R2	S3C5R3	S3C5R4

Before planting, soil samples were taken from each pot, from a depth of 0-5 cm. Five seeds were planted per pot at the depth specified by the seed producer. The planting took place at the beginning of April, and after the 45-day vegetation period, all the plants were harvested. After harvesting, the final soil samples were taken from each pot, from a depth of 0-5 cm, for further analysis.

#### Soil and plant analysis

Sample preparation and analyses were carried out at the Research Center for Studies of Food and Agricultural Products Quality, USAMV Bucharest.

The soil samples were dried at room temperature, ground with a laboratory soil grinder and sifted through a 250 µm sieve, in order to prepare for elemental analysis.

After harvesting the spinach samples, they were weighed and measured.

The plant samples were dried to constant mass, ground with the laboratory grinder into fine powder and kept in exicators until analysed.

Heavy metal analysis was performed using ICP-MS technique (Agilent 7700 system). To perform this analysis, the soil samples were subjected to acid digestion with aqua regia (65% HNO<sub>3</sub> and 37% HCl in 3 to 1 proportion). An amount of 0,1 g of each sample was placed in digestion tubes, adding 6 ml of HNO<sub>3</sub> and 2 ml HCl. The digestion was accomplished using a microwave system (Ethos Up), at 180°C for 15 minutes. For plant samples, the digestion was accomplished using 0.1 g of dried sample, 8 ml HNO<sub>3</sub> and 2 ml H<sub>2</sub>O<sub>2</sub>. The digestion tubes were subjected to the same digestion program. The following heavy metals were studied: Cd, Cu, Ni, Pb, Co and Zn.

The obtained data were processed using IBM SPSS statistical software. Duncan's Multiple Range Test at P≤0.05 level was used for significance determination between groups of means of the six variants used in the experiment and also between the three soil types. The bars in the charts represent the means ± SE of each variant. The same letters above each bar means that they are not significantly different.

## RESULTS AND DISCUSSIONS

#### Heavy metals behaviour in soils

Regarding the initial content of heavy metals, excepting Ni that exceeds the alert threshold, all values are slightly above the reference values according to the order 756/1997 for all three types of soil (Table 5).

Table 5. The amount of heavy metals (HM) in initial soils (C0)

Metal	HM content (mg/kg)		
	S1 C0	S2 C0	S3 C0
Cd	0.23	0.32	0.31
Pb	16.40	21.89	20.70
Ni	106.32	105.21	168.29
Co	10.22	10.33	12.43
Cu	17.68	19.24	27.46
Zn	55.53	60.64	98.73

After contamination, the quantities increased in accordance with the established level of contamination. The contamination was managed by irrigation water; therefore, it is possible that some differences of HM content may occur in the collected soil samples.

The initial contamination level (I) is presented in the Figure 2, alongside the values obtained from soil samples collected after the harvest of the spinach crop (F).

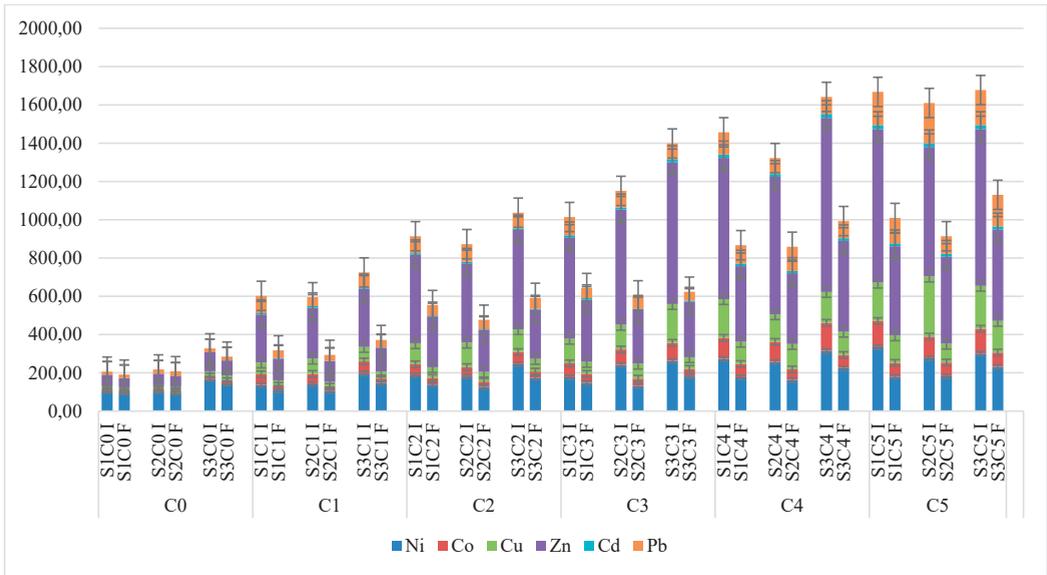


Figure 2. The total amounts of heavy metals in pot soils at the beginning (I) and at the end (F) of the experiment (mg/kg)

As can be seen, excepting the control variant (C0), the level of contamination decreased significantly from the C1 concentration, with the same tendency to the C5 concentration. In

the table below (Table 6) are presented the dropping rates for the heavy metal quantities for each variant.

Table 6. The reduction rates between initial and final pot soil samples, at 0-5 cm depth (%)

Concentration	Soil type	HM quantity reduction between initial and final samples (%)						Average HM loss by each soil type and contamination (%)
		Ni	Co	Cu	Zn	Cd	Pb	
C0	S1	8.72	-0.46	1.09	14.72	7.19	-11.15	<b>3.35</b>
	S2	8.54	5.28	-9.36	5.51	27.57	-9.07	<b>4.74</b>
	S3	14.19	1.82	-3.29	19.67	15.23	2.152	<b>8.29</b>
C1	S1	20.69	56.19	54.02	54.84	62.11	55.01	<b>50.48</b>
	S2	25.32	57.40	68.57	59.52	72.73	32.94	<b>52.75</b>
	S3	25.15	63.65	51.23	59.24	70.18	50.91	<b>53.39</b>
C2	S1	25.72	39.29	49.27	42.58	40.63	38.67	<b>39.36</b>
	S2	30.59	41.63	60.18	47.14	51.90	46.49	<b>46.32</b>
	S3	28.81	49.44	44.68	50.22	44.026	30.73	<b>41.32</b>
C3	S1	16.23	36.17	51.19	38.56	34.43	44.20	<b>36.80</b>
	S2	45.35	53.09	39.46	53.59	31.95	18.66	<b>40.35</b>
	S3	30.93	57.81	70.24	60.98	61.04	42.27	<b>53.88</b>
C4	S1	35.17	36.86	42.52	46.48	24.49	17.08	<b>33.77</b>
	S2	36.96	46.19	8.21	48.48	26.13	-52.76	<b>18.87</b>
	S3	28.60	53.09	24.74	47.74	35.74	-0.94	<b>31.49</b>
C5	S1	46.10	47.38	28.18	41.81	33.61	23.57	<b>36.77</b>
	S2	34.08	36.46	68.72	32.89	21.08	56.16	<b>41.56</b>
	S3	22.89	42.58	26.29	41.74	24.44	9.54	<b>27.91</b>
<b>Average loss for each metal (%)</b>		<b>26.89</b>	<b>40.21</b>	<b>37.55</b>	<b>42.544</b>	<b>38.03</b>	<b>21.91</b>	

Considering that the sampling was done at the depth of 0-5 cm, it means that the heavy metals

migrated to the base of the pots, with the application of successive irrigations carried out

during the vegetation period. This leaching process is dependent on the characteristics of the soil, which makes the modelling efforts very difficult.

There is a significant difference between C0 HM leaching and any of contaminated variants (C1-C5). This means that in the case of low concentrations of heavy metals, they are bound in stable compounds and adsorbed in the soil structure. When the concentration increases, these elements are in excess of the possible bonds and compete with the major elements such as Ca, Mg, Na, K. If they form insoluble compounds, the physical adsorption and chemisorption processes occurs pH dependent and only temporarily reduce the concentration of heavy metals.

Important amounts of heavy metals can be retained in the crystal lattice of silicates, depending of soil type, and are practically harmless to the plant.

As can be seen, there are differences on heavy metals loss, both between soil types and between metal types. It can be noted that the lowest loss was experienced by lead and the highest loss was attributed to zinc. All these differences are generated by the soil characteristics, and they lead to a different response to the plant. This response is influenced by both the type of soil and the remaining amounts of active metals and possibly toxic to plants (Figure 3).



Figure 3. Spinach development on each concentration and soil type (from left to right: S1<C0 to C5>; S2<C0 to C5>; S3<C0 to C5>)

In a previous study, it was shown that the same amounts of heavy metals as those used in this experiment have a significant effect on germination and viability of spinach plants. At the maximum concentration, the viability of seedlings tends to zero, after just ten days (Mot et al., 2019).

Regarding the growth in the pots, after 45 days of vegetations, the following amounts of dried plant were obtained for each concentration and soil type (Table 7).

Table 7. The total dried mass harvested from each soil type and concentration

Soil Type	Dried mass of plant (g)					
	C0	C1	C2	C3	C4	C5
S1	1.9	3	1.3	0	0	0
S2	2.4	3.1	5.2	0	0	0
S3	24.6	18.8	18.7	19.6	7.2	8.3

Apart from the fact that much smaller amounts of plants were obtained from the soils S1 and S2, at the concentrations above C2 the plants did not even survive.

Surprisingly, and the total amounts of heavy metals in S3 are slightly higher, the plants have grown much better, even at high concentrations, including C5, in which germination has been inhibited.

One of the important factors that generated this behaviour is pH. After some authors, this indicator is the main factor that controls the adsorption of cations in the soil.

It has been established that heavy metals form hardly soluble compounds in alkaline media (S3 soil, pH=7,8) and can form precipitates of metal oxides, hydroxides, carbonates and phosphates. Also, the stability of metal complexes derived from organic ligands increases with pH, thus binding heavy metals into stable compounds.

Regarding the accumulation of heavy metals by spinach leaves, this differs both depending on the type of soil and the used concentration.

#### *Cadmium accumulation*

For the same used concentration, a significant difference can be observed in the accumulation of cadmium in different type of soils. Although higher amounts of cadmium were determined in S3 soil, it was not absorbed by the plant. For equivalent concentrations it was observed that the control variant (C0) accumulated an amount 77.72% lower than S1 and 67.7% lower than S2. Also, at concentrations C1 and C2, the spinach accumulated significantly smaller amounts on S3 soil compared to S1 and S2 soils (Figure 4). However, cadmium did not cause the plant death at higher concentrations, in S1 and S2 soils. As proof, in the case of S3 soil, the plants developed up to a concentration of 37.64 mg/kg, reached at C5 level of contamination, almost twice compared to accumulation on S1 and S2 at C2 concentration.

As another observation, the accumulation of Cd for both S1 and S2 at C2 concentration did not exceed the value from C1 concentration. This behaviour may be due both to the existence of a competition of cadmium with other elements, and to the fact that the absorption was inhibited by certain physiological processes.

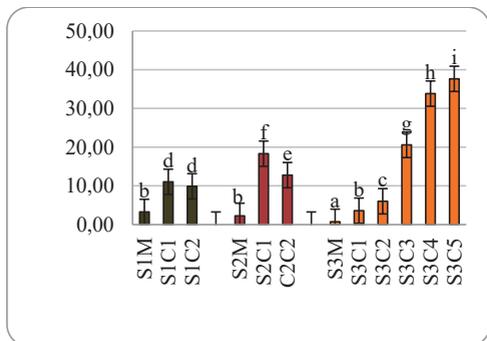


Figure 4. Cd accumulation by spinach on each soil type and concentration (mg/kg)

#### Lead accumulation

Lead accumulation was rather constant on all soils and concentrations, with no significant differences, with two exceptions. The control variant on S3 soil had the lowest concentration in plant leaves, and the accumulation at C5 concentration level, on S3 as well. It should also be mentioned that the amount of heavy metals accumulated by the plant in soil C3 at level C5 does not differ from the amount accumulated by the plant at level C2 in soils S1 and S2 (Figure 5).

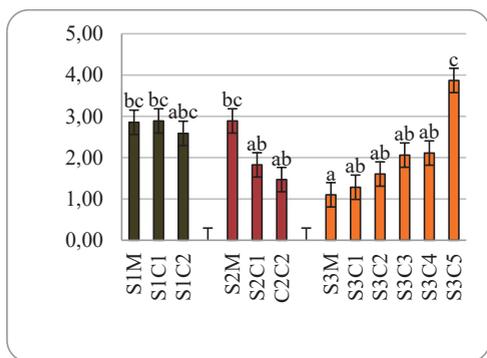


Figure 5. Pb accumulation by spinach on each soil type and concentration (mg/kg)

The results obtained for lead accumulation were somewhat to be expected. Many studies

have found that immobilizing lead leads to low absorption, due to the rapid reaction with the present anions, anions that form hardly soluble compounds. Also, in soils where the soil reaction exceeds pH 6, lead is easily adsorbed by clays or precipitated as carbonates. Here is the case of S3 with alkaline reaction, where the accumulation of lead in plants was lower compared to other soils.

The same observation as on cadmium accumulation, the accumulation of Pb for both S1 and S2 at C2 concentration were lower than C1 concentration, but this is due to the fact that the existence of a high concentration of salts favored the reaction of lead with the anions from soil solution forming insoluble compounds.

#### Nickel accumulation

The accumulation of nickel in spinach plants had a totally different pattern than lead and cadmium. If at low values of soil concentration, no significant difference was observed in the absorption of this metal, with the increase of soil concentration the absorbed amount increased rapidly on S1 and S2. Comparing the value from S3, C2 concentration with values from S1 and S2 with the same C2 concentration, it is observed that the nickel absorption was multiplied by 11.07 and 13.85 respectively in the case of these two soils. The nickel accumulation on S3 gradually increased with soil concentration, but not at the rate of the other soils (Figure 6).

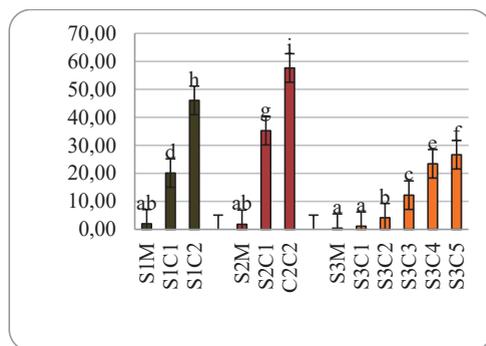


Figure 6. Ni accumulation by spinach on each soil type and concentration (mg/kg)

#### Cobalt accumulation

The cobalt accumulation follows the same pattern as nickel. At low concentrations as

control variants, there were no significant differences between soil types. If the concentration was increased, there were significant differences in absorption between soil types, even from the C1 concentration (Figure 7).

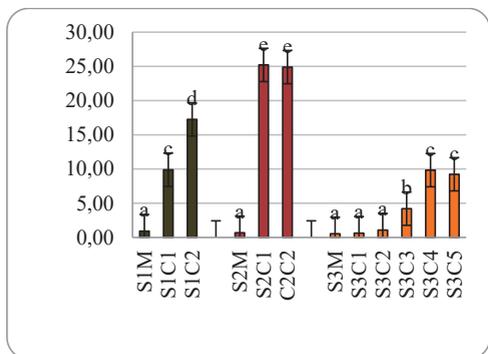


Figure 7. Co accumulation by spinach on each soil type and concentration (mg/kg)

As nickel, at C2 concentrations, the quantity of absorbed heavy metals on S1 and S2 was multiplied by 15,68 and 21,40 respectively.

#### Copper accumulation

The copper absorption did not show significant differences between similar concentrations on S1, S2 and S3 soils, excepting the control variant, when the absorption was greatly influenced by the initial load of heavy metals (Figure 8).

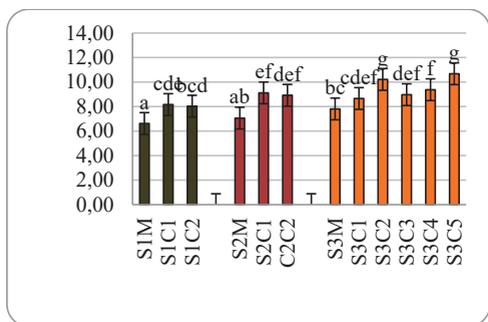


Figure 8. Cu accumulation by spinach on each soil type and concentration (mg/kg)

At greater concentrations (C3, C4, C5), there is a significant increase in copper accumulation in soil S3 compared to S1 and S2. Considering the

contamination with a much larger amount of zinc compared to copper, it is possible that its absorption was limited by the zinc abundance. However, the level of copper absorbed by the plant is below the level required for normal growth. Certainly, the increased level of the other metals inhibited the absorption of copper, resulting in a deficiency in this element.

#### Zinc accumulation

The zinc accumulation, as well as nickel and cobalt, was significantly higher in S1 and S2 soils, compared to S3, at all tested concentrations (Figure 9).

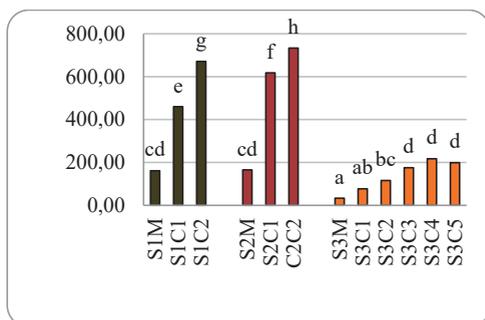


Figure 9. Zn accumulation by spinach on each soil type and concentration (mg/kg)

As some studies show, the soil pH has a great influence on zinc solubility. With the decrease of the pH value, the zinc solubility increases, becoming available in large quantities for plant absorption (Lindsay, 1979). Is the case of S1 and S2 soils, which showed a much higher availability of zinc compared to S3.

Also, some study showed antagonistic interaction of zinc with different elements: Zn-Fe, Zn-Cu, Zn-Ca (Vrinceanu et al., 2010). Therefore, following the absorption of copper on the three soils, a relationship of inverse proportionality can be observed in relation to zinc accumulation.

As an overview, Figure 10 shows the total amount of heavy metals accumulated in the spinach plants that have reached maturity. As can be seen, the amount of absorbed heavy metal keeps the proportionality of the contamination amounts.

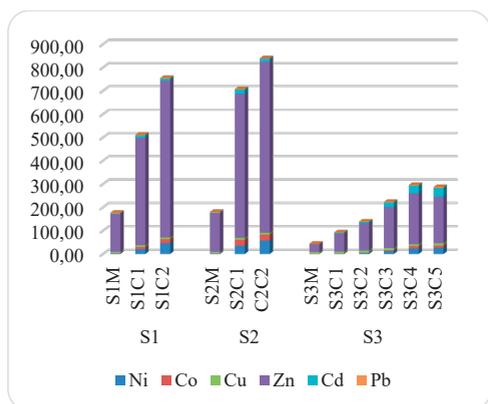


Figure 10. An overview with whole amount of heavy metals accumulated by spinach plants (mg/kg)

## CONCLUSIONS

Except for the control variants, all soils contaminated with all concentrations underwent heavy metal leaching processes, most likely due to excessive amounts of elements that did not find free binding positions, with an average loss over 40%.

The alkaline reaction and the high value of soil OM on fluvisol (S3) have made it possible to grow plants even in conditions of severe soil contamination, due to the capacity of releasing cations and to the high level of binding of heavy metals in complex forms.

The main factor that influenced the absorption of heavy metals by spinach plants was the amount of metal added to the soil. Therefore, zinc was the most accumulated metal, due to massive added amounts (of up to 700 mg/kg).

Soil pH influenced the accumulation of lead in plants, which can be observed in soil with alkaline pH (7.8). The amount of lead was much lower compared to soils with acidic pH.

With an increased abundance of heavy metals in the soil, copper could not be accumulated in the required quantities, even if the amount in the soil was above normal.

By the total amount of plant sample collected, small amounts of heavy metals (below the alert threshold) had a positive influence on plant growth. This is due to the fact that the physiological needs of the plant were covered with microelements, and the amounts of unnecessary elements (Cd, Pb) were not large enough to cause increased toxicity to the plant.

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## METHODOLOGICAL APPROACHES FOR ASSESSING THE DRIP IRRIGATION IMPACT ON THE PEDO-ECOLOGICAL STATE OF IRRIGATED SOILS

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### Abstract

*The paper presents modern approaches to monitoring and assessing the pedological-ecological state of soils under drip irrigation conditions, which includes the features of soil samples selection taking into account the area of the irrigation pipeline and non-irrigated aisles (rows), laboratory-analytical studies of soil samples, calculation of the quantitative characteristics of variation integral indicator of the investigated factors, establishment of a the representative profile / well. Impact assessment of drip irrigation method on the soil processes and the agro- ameliorative state of irrigated lands was carried out according to the soil indicators of the profiles / wells, representative for the studied zones in the rows and the aisle. Proposed approaches allow a reliable assessment of drip irrigation intensity influence on soil processes in order to maintain the pedological-ecological, agro-ameliorative state of the irrigated soils at the safe level, preserve and increase the soil fertility.*

**Key words:** drip irrigation, pedological-ecological state, soil properties, representative profile/well.

### INTRODUCTION

Irrigation is the most powerful factor of human intervention in the natural-ecological environment and a strong factor in soil transformation. Economically and ecologically, drip irrigation technologies are now considered the most productive in agriculture sector. The annual area of irrigated land already reaches 65 - 75 thousand hectares (Шатковський, 2016; Цуркан, 2015). An important feature of land drip irrigation is the introduction of dissolved fertilizers and microelements with irrigation water (fertigation), growth regulators and plant protection products (herbigation, insecticide, fungi, etc.).

With drip irrigation, which is characterized by the local conditions of soil humidity, a rather nature complex of the moisture with salts and nutrients movement dissolved in it, is formed (Lubana & Narda, 2001; Ромащенко, 1998; Чорноземи (2016); Цуркан, 2015, 2018). These processes leads to the manifestation of local agrogenic differentiation of soil properties due to uneven distribution of soil moisture

(Рекомендації, 2012), formation of increased concentration of nutrients (Tsurkan et al., 2018; Шатковський, 2007), changes in the soil salt regime in the places with irrigation water localization (Цуркан, 2018; Ромащенко, 2014), changes in the microbiological soil activity (Венера & Красимов, 2002) and in the agricultural land history use and other factors.

The increased content of nutrients in the fertilizer localization zone persists for a long time and, as a rule, is not limited to one growing season. Therefore, it is difficult to choose a place (area) with the representative soil profile, to obtain objective and correct data for assessing the impact of agricultural activities on the pedological-ecological state of lands under the drip irrigation conditions.

There is a known method of sampling soil under drip irrigation conditions (Балюк et al., 2009), which provides for sampling soil in the area of the irrigation pipeline, crops rows area, on the border of the humidification circuit and in non-irrigated aisles (area). Conducting the analysis of such areas allows one to determine the pedo-ecological soil state in drip irrigation

conditions and predict the yield of agricultural crops.

To study the microdistribution of mineral nutrients from the fertilization lands, the authors (Трапезников et al., 1999) proposed the method including layer-by-layer of soil sampling, in form of horizontal monoliths with size  $2 \times 2$  cm, on perpendicular to the direction of the fertilizer application.

Determination of the optimal points of soil sampling for operational control of the processes of drip irrigation and fertigation, the method was proposed for sampling soil for moisture (by setting tensiometers) and mineral nutrients content at the points of maximum concentration of the root system in the middle of the distance between the irrigation pipeline and the border of the moisture zone in the plant budding phase at depth of 20 cm, and then at 40 cm (Лимар & Кашеев, 2011; Tsurkan et al., 2018, 2019).

Under the conditions of local application of liquid and gaseous nitrogen fertilizers, was proposed to evaluate the change in the parameters of soil fertility indicators by taking soil samples, avoiding the formation of parallel or perpendicular lines that coincide with the place of fertilization (tape) and equal distances between spot samples. Thus, 20 individual soil samples are taken from an elementary site from a depth of 20 cm, which are thoroughly mixed under laboratory conditions and a mixed average soil sample is formed (Мирошниченко et al., 2016).

The generally accepted methods of pedoagrochemical survey and assessment of the irrigated lands state do not allow for an objective assessment of soil fertility indicators in cases of local application of fertilizers due to an increase in the spatial variability of many of them, which increases the likelihood of taking an insufficiently representative sample and affects the reliability of the research results.

Consequently, studies should refer to a specific profile or well, which would be representative (typical) for this study object. Typically, the representative profile ( $P_{typ.}$ ) is considered to be the profile or well, which the values of the studied factors deviate least from the average group of these factors. Moreover, as a rule, the effective factors are compared. To identify the changes that occur in the soil under the drip

irrigation influence, comprehensive studies are carried out, then the selected profile should be average or representative (typical) not for one indicator, but for a set of various indicators of the soil.

Thus, the optimal way to assessing the impact of drip irrigation on the pedological and ecological properties of soils is to establish representative profiles / wells for irrigated and non-irrigated (space between rows) zones according to factorial characteristics. To do this, it is necessary to taking into account the close relationship of the factors-arguments with the effective indicator, identifying the quantitative influence of the factors-arguments on the effective indicator, according to the ecological indicators of which the impact on soil processes of the drip irrigation method is assessed. In this regard, the purpose of our research is to develop modern approaches to monitoring the state of the soil cover under the drip irrigation conditions.

## MATERIALS AND METHODS

The research was carried out on the experimental fields of "Dobra Gorodina", Belyaevsky district, Odessa region. The field experiment was laid on the southern chernozem, low-humiferous, clayey-loamy in conditions of drip irrigation and vegetable crops rotation. The farm has been using a drip irrigation system for watering vegetable crops since 1996-1997. Alternation of vegetable in crop rotation: tomatoes - sweet peppers - onions (2 years) - spring barley (2 years).

For irrigation, the water from Dniester River was used. Irrigation water is characterized by the following properties: hydrocarbonate - calcium composition, fresh (mineralization  $< 0.5$  g/dm<sup>3</sup>),  $pH_{H_2O} = 7.5-7.8$ , concentration of toxic ions - 4 mmol·eq/dm<sup>3</sup>. According to agronomic indicators, the irrigation water belongs to the I quality class, and it is suitable for irrigation without restrictions. In order to optimize the nutritional regime with irrigation water (in the vast irrigations majority), dissolved fertilizers and microelements are applied. Mineral fertilizers are represented by phosphorus, nitrogen, potash - both simple and complex. The pre-irrigation soil moisture is maintained at the level of 80-85 - 70% of the

lowest water-holding capacity after the phases of plant development.

Taking into account the local natural conditions of soil moisture with drip irrigation, soil samples were taken in the area of the irrigation pipeline and in the middle of the non-irrigated zone (row spacing). Soil samples were taken to a depth of 1 m, in layers of 0-10, 10-30, 30-50, 50-70, 70-100 cm. Sampling from the 0-10 cm soil layer is due to the technology of growing seedlings, in which irrigation pipelines are laid into furrows to a depth of 10 cm.

In the work field process, the laboratory and analytical studies of soil samples were carried out according to standard certified methods, followed by statistical processing, which included the determination of the salt composition of the water extract, the physico-chemical properties of soils, the content of humus, mineral nitrogen, mobile phosphorus and exchangeable potassium by the Machigin method.

The analyzes were carried out with STATISTICA 7.0 (StatSoft Inc., USA). Graphs and diagrams were built using MS Excel 2010 (Microsoft Corp., USA) and STATISTICA 7.0 (StatSoft Inc., USA).

## RESULTS AND DISCUSSIONS

As a result of pedological and agrochemical examination of chernozems under drip irrigation conditions, during the soil samples selection in the area of the irrigation pipeline and in the middle of the non-irrigated zone (row spacing), it was found that statistically processed indicators of the most important agricultural production characteristics of the soil differed significantly in the upper layer of 0-50 cm. Within the experimental plot and along the soil profile (in deep), the parameters of the content of chlorides, sulfates, sodium, nitrate and ammonia nitrogen, mobile phosphorus, carbonates had a high coefficient of variation ( $V > 25\%$ ).

The calculated variation coefficients for above parameters were characterized by the high variability both in the rows and in the aisles. That is, the use of drip irrigation causes a significant increase in the heterogeneity of soil properties within the field and in deep of the profile, which leads to a variety of properties and their agro-ameliorative state.

As our research has shown, the residual effect of previous years of soil moisture and fertilization when growing vegetable crops, affects the state of sowing spring barley (a catch crop in a drip-irrigated vegetable crop rotation). The diversity of sowing barley is manifested in a higher plant height, and especially in a better formed ear in the place of the former rows.

Based on the studies carried out, we believe that in order to assess the ecological impact of drip irrigation on the soil properties of irrigated soils, it is necessary to establish profiles / wells representative for the zones of the irrigation pipeline and the non-irrigated zone (row spacing), which will increase its reliability and objectivity.

Proceeding from the fact, that changes in humus content are not only directly related to changes in all soil properties, but also clearly reflect the influence of external processes, we have taken the humus content ( $y$ ) as an effective factor. Humus is an integrating indicator that determines most of the properties of soils and their overall fertility (Baliuk et al., 2020). At the same time, it reacts rather quickly to external influences of a natural and, mainly, anthropogenic nature, being a reliable indicator of changes occurring in the soils. Soil indicators were taken as factors-arguments ( $x_j = 1, 2, 3, \dots, 11$ ) are presented in Table 1.

Thus, the correlation task with 11 factorial features was compiled. The coefficient of multiple determination  $R^2$  turned out to be 0.96 for irrigation pipeline zone, and 0.99 for the non-irrigated zone, that is, the specific weight of the combined effect of the selected factors on the humus content is 96% and 99%, respectively.

The number of population units for the test line under the irrigation pipeline and non-irrigated area:  $j = 40$  ( $p = 8$  wells, in each of which soil samples were taken from the depths ( $k = 5$ ): 1 - 0-10 cm; 2 - 10-30 cm; 3 - 30-50 cm; 4 - 50-70 cm; 5 - 70-100 cm).

The deviation from the ratio unit of the quantitative value of each factor  $x_i$  ( $i = 1, 2, 3, \dots, 11$ ) to its average value for the entire number of aggregate units ( $j = 1, 2, 3, \dots, 40$ ) was determinate (1):

$$\delta_{ij} = \left| 1 - \frac{x_{ij}}{\bar{x}_i} \right|, \quad (1), \quad \text{where: } x_{ij} - \text{the factor value } i \text{ in } j - \text{ in the sampling soil;}$$

$\bar{x}_i$  – the average value of the factor  $i$  for the set of soil samples.

Table 1. Results of establishing a representative soil profile/well by a set of factor signs

Factor name	Symbols	Testing line - under the irrigation pipeline		Testing line - in the middle of the non-irrigated area (row spacing)	
		$r_{yx_i}$	$d_{yx_i}$	$r_{yx_i}$	$d_{yx_i}$
Humus, %	Y	-	-	-	-
pH <sub>H2O</sub>	X <sub>1</sub>	-0.653	0.426	-0.685	0.469
HCO <sub>3</sub> <sup>-</sup> , mmol per 100 g of soil	X <sub>2</sub>	0.237	0.056	0.081	0.007
Cl <sup>-</sup> , mmol per 100 g of soil	X <sub>3</sub>	0.111	0.012	0.039	0.002
SO <sub>4</sub> <sup>-</sup> , mmol per 100 g of soil	X <sub>4</sub>	-0.541	0.293	-0.527	0.278
Ca <sup>2+</sup> , mmol per 100 g of soil	X <sub>5</sub>	-0,250	0,063	-0,415	0,172
Mg <sup>2+</sup> , mmol per 100 g of soil	X <sub>6</sub>	-0,037	0,001	-0,457	0,209
Na <sup>+</sup> , mmol per 100 g of soil	X <sub>7</sub>	0.855	0.732	0.814	0.662
K <sup>+</sup> , mmol per 100 g of soil	X <sub>8</sub>	-0.909	0.826	-0.900	0.810
Mineral nitrogen, g per 100 g of soil	X <sub>9</sub>	0.575	0.331	0.418	0.174
Mobile phosphorus, g per 100 g of soil	X <sub>10</sub>	0.847	0.718	0.837	0.700
Exchangeable potassium, g per 100 g of soil	X <sub>11</sub>	0.951	0.904	0.957	0.915
Multiple determination coefficient	R <sup>2</sup>	-	0.960	-	0.990
Correlation ratio	R	0.980	-	0.970	-

The individual indicator of quantitative characteristics variation of the study factors, which characterizes the sum of linear deviations for all soil sampling ( $j = 1, 2, 3, \dots, 40$ ) distributed on  $p$  groups of wells (1, 2, 3 ... 8) and  $k$  subgroups of sampling depths (1, 2, 3 ... 5) was determined (2).

$$v_{pkj} = \sum_{i=1}^n \delta_{ij} d_i \quad (2), \quad \text{where:}$$

$\delta_{ij}$  – deviation from the ratio unit of the quantitative value of the factors  $x_{ij}$  to its average value  $\bar{x}_i$ , taken in modulus;  $d_i$  – partial coefficient of determination  $i$ -factor. The integral criterion for establishing the typical (representative) well as the sum of individual indicators of the quantitative

characteristics variation of the study factors by the depths of extraction for each well was calculated.

The quantitative value of the integral criterion for establishing the type profile / well of which will be minimal, is the typical (representative) for the irrigation pipeline zone and non-irrigated zones. So, in our example, the criterion minimum value for setting the typical profile / well according to the research results is observed for irrigation pipeline zone along the well  $p_2$ , and for the of non-irrigated row spacing zone -  $p_{10}$  (Table 2).

Thus, the  $P_{typ}$ . for the irrigation pipeline area there is  $p_2$ , and for non-irrigated row spacing area -  $p_{10}$ . Soil indicators of these wells have the smallest deviation from the average group value in terms of a set of factor signs, that is, they are representative of the studied zones. In

addition, the individual values of the criterion for the sampling depth do not exceed the average values for the corresponding sampling

depth which makes it possible to take into account the distribution of indicators along the soil profile (Table 2).

Table 2. Calculated values of individual indicators of studied factors quantitative characteristics and an integral criterion for determining the typical soil profile/well

No. of subgroup depths Soil sampling	k <sub>1</sub>	k <sub>2</sub>	k <sub>3</sub>	k <sub>4</sub>	k <sub>5</sub>	$\sum_{k=1}^5 v_{pkj}$
	$v_{pkj} = \sum_{i=1}^n \delta_{ij} d_i$					
<i>Sampling line - under the irrigation pipeline</i>						
p <sub>1</sub>	1.26	1.10	1.26	1.01	0.95	5.58
p <sub>2</sub>	<b>0.76</b>	<b>0.87</b>	<b>0.81</b>	<b>0.87</b>	<b>0.93</b>	<b>4.30</b>
p <sub>3</sub>	0.85	0.91	0.96	0.90	0.93	4.56
p <sub>4</sub>	1.12	1.13	0.95	1.00	0.94	5.13
p <sub>5</sub>	1.02	1.00	0.96	0.87	0.90	4.75
p <sub>6</sub>	1.25	1.05	0.82	0.91	0.91	4.94
p <sub>7</sub>	0.91	1.14	0.89	0.93	0.91	4.77
p <sub>8</sub>	1.01	0.82	0.89	0.87	0.83	4.42
$\overline{v_{kj}}$	1.02	1.00	0.94	0.92	0.91	-
<i>Sampling line - in the middle of the non-irrigated area (row spacing)</i>						
p <sub>9</sub>	1.13	1.56	0.99	0.98	1.01	5.67
p <sub>10</sub>	<b>0.91</b>	<b>0.96</b>	<b>0.99</b>	<b>0.94</b>	<b>0.94</b>	<b>4.74</b>
p <sub>11</sub>	0.99	1.18	0.91	0.99	0.99	5.06
p <sub>12</sub>	1.10	1.43	0.98	0.95	0.90	5.35
p <sub>13</sub>	0.85	1.40	0.97	0.94	0.90	5.06
p <sub>14</sub>	1.01	1.22	0.92	0.98	0.94	5.07
p <sub>15</sub>	1.11	1.49	0.97	0.97	1.00	5.53
p <sub>16</sub>	1.17	1.44	1.01	1.01	1.05	5.68
$\overline{v_{kj}}$	1.03	1.33	0.97	0.97	0.96	-

The method to assess the impact of the drip irrigation and the agro-ameliorative state of irrigated lands under drip irrigation conditions on soil processes need to carry out using the soil indicators of representative profiles / wells for the studied representative zones.

Along with the deviation of each factor from its average group value, the proposed criterion takes into account the qualitative and quantitative dependence of the factors-arguments and the effective signs. The proposed criterion for establishing the representative profile / well takes into account the deviation of the factor-arguments from their average values, taking into account the tightness of each relationship of the factor attributes -  $x_i$  with the effective attribute -  $y$ .

Assessment of the drip irrigation impact on soil processes and the agro-ameliorative state of

irrigated lands under drip irrigation conditions along representative profiles/wells for irrigation pipeline zones and non-irrigated row spacing allows obtaining more information on the land and soil regimes, processes, properties and dynamics of their changes. The proposed approaches make it possible to reliably assess the intensity of drip irrigation influence on soil processes in order to maintain the ecological and agro-ameliorative state of irrigated soils at a safe level, preserve and increase their fertility.

## CONCLUSIONS

In drip irrigation conditions for objective and correctly assessment of its influence on the soil processes and the agro-ameliorative soils state, the soil sampling zone is of no small importance. Therefore, when conducting an agro-ameliorative survey of soils under drip

irrigation conditions, it is recommended to take soil samples separately, in the irrigation pipeline zone and in the middle of the non-irrigated zone (row spacing).

Given the high variability both in the rows and in the row-spacing of statistically processed indicators of the most important agro-production characteristics, it is mandatory to establish profiles/wells representative for the irrigation pipeline and the non-irrigated (row spacing) zones. The drip irrigation impact on the pedological-ecological state is assessed by soil indicators of profiles/wells representative for the studied zones.

Thus, the proposed approach is more objective, mathematically reliable, since the influence of the drip irrigation method and the agromeliorative state of irrigated lands are assessed by pedo-ecological indicators of representative profiles/wells for the irrigation pipeline and the non-irrigated zones. Consequently, conducting pedo-ecological monitoring of the soils state in drip irrigation conditions and evaluating its effect according to the proposed method is suitable for all agricultural crops, without exception, and any schemes for using drip irrigation.

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## METHODS FOR SEEDBED PREPARATION IN FORESTRY NURSERIES

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### Abstract

*This paper presents the results of our research regarding the six methods for seedbed preparation in nursery of the West of Romania. This method are: Vibrocombinator equipped with Gama type active organs (Vibro\_Gama), Vibrocombinator equipped with Delta1 type active organs (Vibro\_Delta1), Vibrocombinator equipped with Delta2 type active organs (Vibro\_Delta2), Disc harrow (Disks), Cultivator (Cultivator) and Rotary harrow (Rotary harrow). In order to evaluate the most efficient method of preparing the germination bed, the following physical-mechanical properties of the soil were determined: moisture, bulk density, total porosity and soil compression degree and water retention. In order to carry out the research, we settled a nursery of the West of Romania so that we could have six methods for seedbed preparation. From each profile was collected soil samples in three steps of 5, 10 and 15 cm. For each sample six repetitions were performed (N = 6). We started by measuring the particle size distribution (granulometric composition) and the main physical properties of the soil. The advantages of using vibro-combinators are: perfect preparation of seedbed in difficult working conditions and preservation of soil moisture. Such important factors can ensure fast, uniform and early germination of seeds, these requirements standing at the basis of abundant harvests. The research investigated the soil tillage performances and the environmental impact of several active elements, at certain soil depths.*

**Key words:** Vibro-combinator, disc harrow, rotary harrow, cultivator, bulk density, total porosity, compression degree.

### INTRODUCTION

Seedbed structure directly or indirectly affects crop establishment by modifying seed-soil contact, acting as mechanical obstacles or modifying temperature, moisture and oxygen contents of seedbed as well as the dynamic of pests, pathogens and weeds. However, very few detailed descriptions of seedbed's structure of major field crops exist to date, in terms of precise aggregate size distributions in relation to different factors including the cropping system, soil and climatic conditions and their interactions (Lamichhane et al., 2021).

Seedbed structure can significantly affect early growth and development of the crop, by altering abiotic components of the seedbed including soil-plant contact, mechanical forces exerted on plant or plant parts, soil aeration, thermal regime and water balance (Dexter,

1988; Dexter, 2004a; Dexter, 2004b; Boja et al., 2012; Boja et al., 2013).

In addition, seedbed structure can also affect the impact of early biotic stresses and the effectiveness of weed control (Glen et al., 1989; Bale et al., 1992; Otten et al., 2006; Finney et al., 2008; Melander et al., 2011; Boja et al., 2018a).

A seedbed containing a high proportion of large soil aggregates not only leads to a poor seed-soil contact but it also cools down more rapidly thereby slowing down the seed imbibition and germination process (Brown et al., 1996; Håkansson et al., 2002; Boja et al., 2016; Boja et al., 2020).

Consequently, the time interval between the seed germination and seedling emergence phase in a seedbed comprising bigger soil aggregates is longer, due to the increased tortuosity of the seedling path before reaching the soil surface (Boiffin et al., 1992).

Likewise, the risk of seedling death before emergence is higher in coarser seedbeds as seedlings can be trapped under the soil aggregates encountered during its elongation from the sowing depth (Dürr et al., 2000).

Delayed emergence also increases the risk of seedling death in an indirect way; for example, by increasing the probability of attacks due to soil-borne pests and pathogens owing to longer heterotrophic phase or topsoil crust formation impeding the emergence (Gallardo-Carrera et al., 2007).

A previous study (Boizard et al., 2002), based on a field experiment, investigated cumulative effects of cropping systems on the structural state of the tilled layer, in particular the proportion of compacted zones in a loamy soil that is characteristic of northern European soils. The authors showed that the compaction level of a soil was dependent on the soil moisture at the time of field operations as well as the characteristics of the machinery used and that there was no indication of irreversible cumulative degradation.

Another study (Boizard et al., 2013; Boja et al., 2016; Boja et al., 2018b), compared the impact of conventional versus reduced tillage on the soil structure evolution and showed that the soil structure in the untilled layer mainly depends on the soil compaction intensity and that regeneration of this compacted layer over time was slower compared with that of the tilled layer.

Nursery seedbed characterization is not only time consuming and resource intensive but also difficult to perform due to limited field access, especially under rainy seasons and high moisture conditions.

Only little knowledge is available to date concerning precise numerical data characterizing seedbed structure and its variations (Braunack et al., 1989; Braunack et al., 1991; Aubertot et al., 1999; Hammer et al., 2001; Gallardo-Carrera et al., 2007).

Together with the action of climatic nature, the soil, as a system, suffers from the influences of mechanical nature, related on one hand to the tillage process and, on the other hand, to the passing of equipments. According to the

characteristics of the tools used and of the exploitation conditions, the first ones are extremely diverse, being conceived to fragmentize and break up the superior part of the soil.

The passing of the equipment represents another way of destroying the texture of the soil, and to favour the apparition of the compaction phenomenon, at some point, in unfavourable climatic conditions, imposed by the cultural calendar (Boja et al., 2018c; Vidrean et al., 2018).

## MATERIALS AND METHODS

This paper presents the results of our research regarding the six methods for seedbed preparation in Iarac forestry nursery (Figure 1 and Table 1) of the West of Romania. This methods are: Vibrocombinator equipped with Gama type active organs (Vibro\_Gama), Vibrocombinator equipped with Delta1 type active organs (Vibro\_Delta1), Vibrocombinator equipped with Delta2 type active organs (Vibro\_Delta2), Disc harrow (Disks), Cultivator (Cultivator) si Rotary harrow (Rotary harrow) (Figure 2).

In order to evaluate the most efficient method of preparing the germination bed, the following physical-mechanical properties of the soil were determined: moisture, bulk density, total porosity, soil compression degree and water retention. In order to carry out the research, we settled a nursery of the West of Romania so that we could have six methods for seedbed preparation.

From each profile was collected soil samples in three steps of 5, 10 and 15 cm. For each sample six repetitions were performed (N=6). We started by measuring the particle size distribution (granulometric composition) and the main physical properties of the soil.

There were taken samples in the natural settlement with metallic cylinders of 100 cm<sup>3</sup>, in order to determine the physical properties at three levels in depth (0-10; 10-20; 20-30 cm); for each sample, the sampling was repeated six times, after the execution of each technical work.



Figure 1. The placement of the Iarac nursery

Table 1. The description of the nursery included in the experiment

Nursery	Altitude (m)	Zone of vegetation	Climate province		Average annual rainfall (mm)	Soil type
			Köppen	Stoenescu		
Iarac	100	Forest steppe	C.f.a.x.	I.B.p.1	500-600	Alluvial (vertic-gleyed)



Vibro\_Gama



Vibro\_Delta1



Vibro\_Delta2



Disks



Cultivator



Rotary harrow

Figure 2. Methods for seedbed preparation in nursery

## RESULTS AND DISCUSSIONS

The bulk density is one of the main indicators of the settlement of the soil and also one of the

determining factors of some of the properties of the soil. High values of the bulk density signify the decrease of the capacity to retain water, of the permeability, of aeration and the increase of

the mechanical resistance opposed by the soil at works and moreover at the penetration of the roots; low bulk density can reduce sometimes the bearing, making difficult the traffic and the execution of the processing works of the germination bed (Boja et al., 2012).

The porosity (the lacunar space) registers higher values while the content of the soil grows in organic matter and offers some important indications in relation with some of the properties of the soil. Thus, high values indicate a high capacity to retain water (Boja et al., 2018a).

The absolute values of the bulk density or of the total porosity cannot be interpreted accordingly in order to appreciate the state of settlement of the soil, because their practical significance is very different from soil to soil according to their texture (Boja et al., 2016).

The determination of the settlement of the soil is well taken by using a synthetic indicator which shows that the compression level and the

deficit of total porosity are met. The indicator which includes the bulk density (total porosity) and takes into account the soil texture is the compression degree (Vidrean et al., 2018).

Apart from its significance as general indicator of its state of settlement, the compression degree practically reflects the state of breaking up and compression of the soil. In certain situations, the elimination of the soil compaction is difficult to be carried out, but it is possible to minimize it through the proper soil management. It is easier to avoid the compaction rather than to eliminate it after its installation, because the correction measures can be expensive and cannot totally solve the problem.

The results of the research are presented though average values according to the granulometric analysis in Table 2. When analysing the granulometric curves, one can notice the fact that there was a sandy-dusty-clay-like texture.

Table 2. Average values of the granulometric analysis

Depth of prelevation		Values of the granulometric analysis				
		Sand, %		Dust, %		Clay, %
		Coarse	Fine	I	II	
cm		>0.2	0.2-0.02	0.02-0.01	0.01-0.002	<0.0002
%						
NURSERY IARAC						
Depth	0-10	1.7	39.0	14.5	24.2	20.6
	10-20	1.8	37.5	14.1	23.0	23.8
	20-30	2.4	39.5	14.5	18.5	25.2
Average on profile		2.0	38.7	14.3	21.9	23.2

To synthesize more efficiently the data taken and to be able to describe completely the intrinsic characteristics of the sample, it was chosen a statistic processing with the aid of the program KyPlot (<http://www.kyplot.software.informer.com>). The results obtained are given in Tables 3-6, having as a purpose to underline

the variance a six methods for seedbed preparation in nursery. Thus, for each nursery were included in the experiment resulted in fifteen statistical indicators for each technical work, but also witness sample. For example we present the determination of statistical indicators for the depth of 5 cm.

Table 3. Statistical indexes of variation of moisture, depth 5 cm

Statistical indicator	Witness sample CTRL	Methods for seedbed preparation					
		Vibro_Gama	Vibro_Delta1	Vibro_Delta2	Disks	Cultivator	Rotary harrow
Mean	20.04	21.52	21.75	23.83	23.70	19.52	18.53
S.E.M.	0.95	0.06	0.08	0.11	1.39	0.70	0.57
Standard deviation	2.32	0.15	0.19	0.28	3.40	1.70	1.40
Coefficient of variation	0.12	0.01	0.01	0.01	0.14	0.09	0.08
Minimum	16.50	21.30	21.50	23.45	19.84	17.71	17.08
Maximum	23.19	21.70	22.00	24.20	27.72	22.18	20.29
The nr. of feature values	6	6	6	6	6	6	6
Skewness	-0.20	-0.31	0.00	0.00	0.19	0.47	0.12
Curtosis	-0.82	-1.15	-1.27	-1.27	-1.54	-1.11	-1.78
Mean Deviation	2.10	0.14	0.18	0.27	3.30	1.68	1.49

Statistical indicator	Witness sample CTRL	Methods for seedbed preparation					
		Vibro_Gama	Vibro_Delta1	Vibro_Delta2	Disks	Cultivator	Rotary harrow
Median	20.12	21.55	21.75	23.83	23.23	19.28	18.40
Range	6.69	0.40	0.50	0.75	7.88	4.47	3.21
Confidence Level (0,95)	2.43	0.15	0.20	0.29	3.57	1.79	1.47
Lower Confidence Limit	19.09	21.46	21.67	23.71	22.31	18.82	17.96
Upper Confidence Limit	20.99	21.58	21.83	23.94	25.09	20.21	19.10

Table 4. Statistical indexes of variation of bulk density, depth 5 cm

Statistical indicator	Witness sample CTRL	Methods for seedbed preparation					
		Vibro_Gama	Vibro_Delta1	Vibro_Delta2	Disks	Cultivator	Rotary harrow
Mean	1.44	1.39	1.35	1.31	1.43	1.60	1.74
S.E.M.	0.01	0.00	0.01	0.02	0.05	0.10	0.04
Standard deviation	0.02	0.01	0.03	0.06	0.12	0.24	0.10
Coefficient of variation	0.01	0.01	0.02	0.04	0.08	0.15	0.06
Minimum	1.41	1.38	1.31	1.23	1.21	1.31	1.62
Maximum	1.47	1.40	1.39	1.38	1.54	1.84	1.87
The nr. of feature values	6	6	6	6	6	6	6
Skewness	0.24	0.00	0.00	0.00	-1.03	-0.34	-0.01
Curtosis	-0.68	-1.50	-1.50	-1.27	-0.08	-1.61	-1.27
Mean Deviation	0.02	0.01	0.03	0.05	0.10	0.24	0.09
Median	1.44	1.39	1.35	1.31	1.45	1.67	1.75
Range	0.06	0.02	0.08	0.15	0.33	0.53	0.25
Confidence Level (0,95)	0.02	0.01	0.03	0.06	0.13	0.25	0.10
Lower Confidence Limit	1.43	1.39	1.34	1.28	1.38	1.50	1.70
Upper Confidence Limit	1.45	1.39	1.36	1.33	1.48	1.69	1.78

Table 5. Statistical indexes of variation of total porosity, depth 5 cm

Statistical indicator	Witness sample CTRL	Methods for seedbed preparation					
		Vibro_Gama	Vibro_Delta1	Vibro_Delta2	Disks	Cultivator	Rotary harrow
Mean	32.22	46.54	48.17	49.81	44.81	39.97	35.68
S.E.M.	4.11	0.14	0.51	0.88	1.25	4.12	1.47
Standard deviation	10.07	0.34	1.24	2.16	3.06	10.09	3.61
Coefficient of variation	0.31	0.01	0.03	0.04	0.07	0.25	0.10
Minimum	22.10	46.15	46.54	46.92	40.88	28.36	30.56
Maximum	45.22	46.92	49.81	52.69	48.36	51.53	40.06
The nr. of feature values	6	6	6	6	6	6	6
Skewness	0.54	-0.02	0.00	0.00	0.10	0.16	-0.06
Curtosis	-1.48	-1.50	-1.32	-1.27	-1.44	-1.63	-1.22
Mean Deviation	10.12	0.31	1.19	2.08	2.98	10.29	3.38
Median	28.29	46.54	48.17	49.81	44.39	38.31	35.34
Range	23.12	0.77	3.27	5.77	7.48	23.17	9.50
Confidence Level (0,95)	10.57	0.36	1.31	2.27	3.22	10.59	3.79
Lower Confidence Limit	28.10	46.40	47.67	48.93	43.56	35.85	34.21
Upper Confidence Limit	36.33	46.68	48.68	50.69	46.06	44.09	37.15

Table 6. Statistical indexes of variation of soil compaction degree, depth 5 cm

Statistical indicator	Witness Sample CTRL	Methods for seedbed preparation					
		Vibro_Gama	Vibro_Delta1	Vibro_Delta2	Disks	Cultivator	Rotary harrow
Mean	23.19	8.07	4.84	1.61	2.64	18.50	26.87
S.E.M.	6.16	0.28	1.00	1.74	3.79	6.13	3.17
Standard deviation	15.08	0.68	2.46	4.27	9.27	15.01	7.77
Coefficient of variation	0.65	0.08	0.51	2.65	3.51	0.81	0.29
Minimum	3.79	7.31	1.61	-4.09	-14.28	-0.02	17.22
Maximum	40.61	8.83	8.07	7.31	10.72	35.09	37.32
The nr. of feature values	6	6	6	6	6	6	6
Skewness	-0.36	0.00	0.00	0.00	-1.04	-0.13	-0.07
Curtosis	-1.44	-1.50	-1.32	-1.27	-0.06	-1.72	-1.31
Mean Deviation	14.64	0.61	2.36	4.10	7.71	15.77	7.29
Median	27.49	8.07	4.84	1.61	4.07	20.59	28.03

Statistical indicator	Witness Sample CTRL	Methods for seedbed preparation					
		Vibro_Gama	Vibro_Delta1	Vibro_Delta2	Disks	Cultivator	Rotary harrow
Range	36.82	1.52	6.46	11.40	25.00	35.11	20.10
Confidence Level (0,95)	15.83	0.71	2.58	4.48	9.73	15.75	8.16
Lower Confidence Limit	17.03	7.79	3.84	-0.13	-1.14	12.38	23.69
Upper Confidence Limit	29.34	8.35	5.84	3.35	6.43	24.63	30.04

Analyzing the average values of the six variants of seedbed preparation, for the five physical-mechanical properties of the soil, for the sampling depth of 5 cm, the best values were obtained when using the Vibrocombinator equipped with active organs of Delta2 type (Vibro\_Delta2).

The mechanical processing of the soil through traditional and modern methods is currently put under question due to the high energy consumption and the continuous degradation of the arable horizon through erosion and excessive compaction.

Analyzing the variation of soil moisture on the three sampling depths, it is noted that at 5 cm depth was recorded the maximum soil moisture after processing with Vibro\_Delta2 and at 10

and 15 cm depth the maximum soil moisture accumulated was obtained after processing with Vibro\_Delta1 (Figure 3).

The lowest bulk density were recorded after processing with Vibro\_Delta2 (5 cm) and Disks (10-15 cm) (Figure 4).

Total porosity recorded maximum values for all depths after the preparation of the germination bed with Vibro\_Delta2 (Figure 5).

The soil compression degree recorded the lowest values when preparing the germination bed with Vibro\_Delta2 (5 cm) and Disks (10-15 cm) (Figure 6).

The water retention reached maximum values when preparing the germination bed with the help of Vibro\_Delta2 (5 cm) and Vibro\_Gama (10-15 cm) (Figure 7).

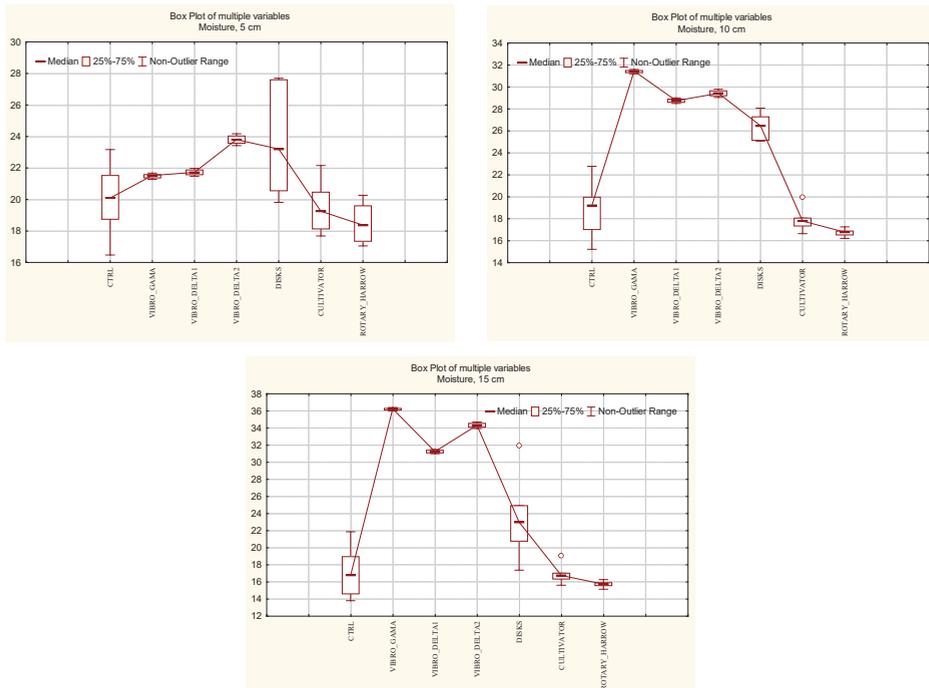


Figure 3. Variation of soil moisture for seedbed preparation, for three depth

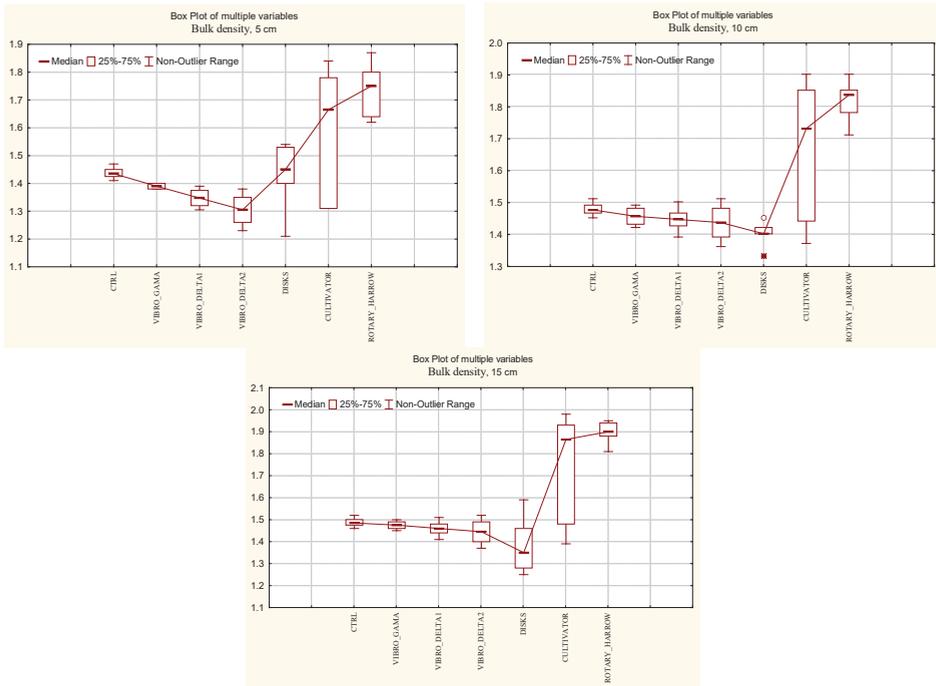


Figure 4. Variation of bulk density for seedbed preparation, for three depth

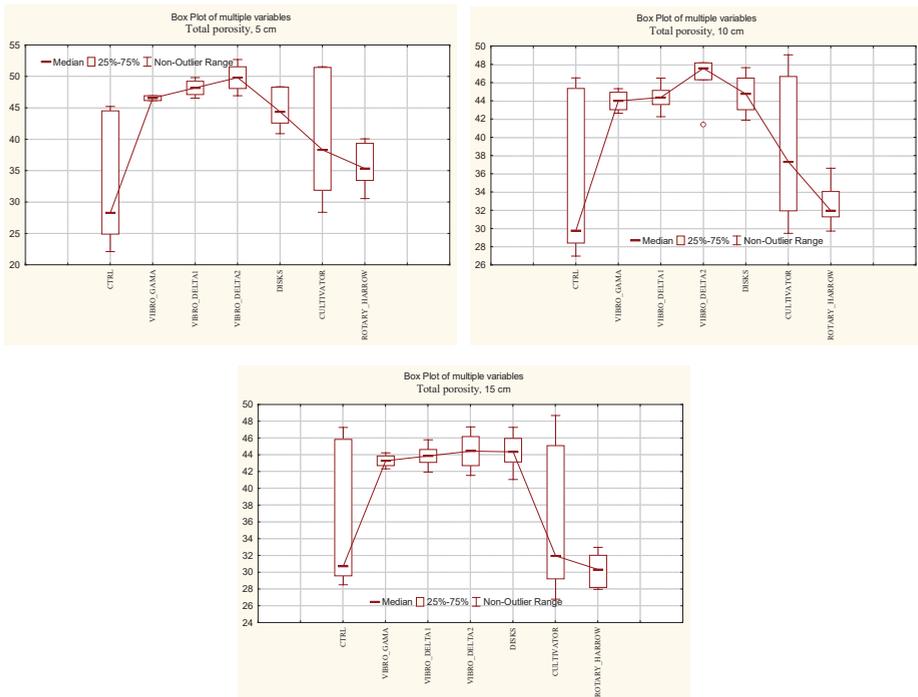


Figure 5. Variation of total porosity for seedbed preparation, for three depth

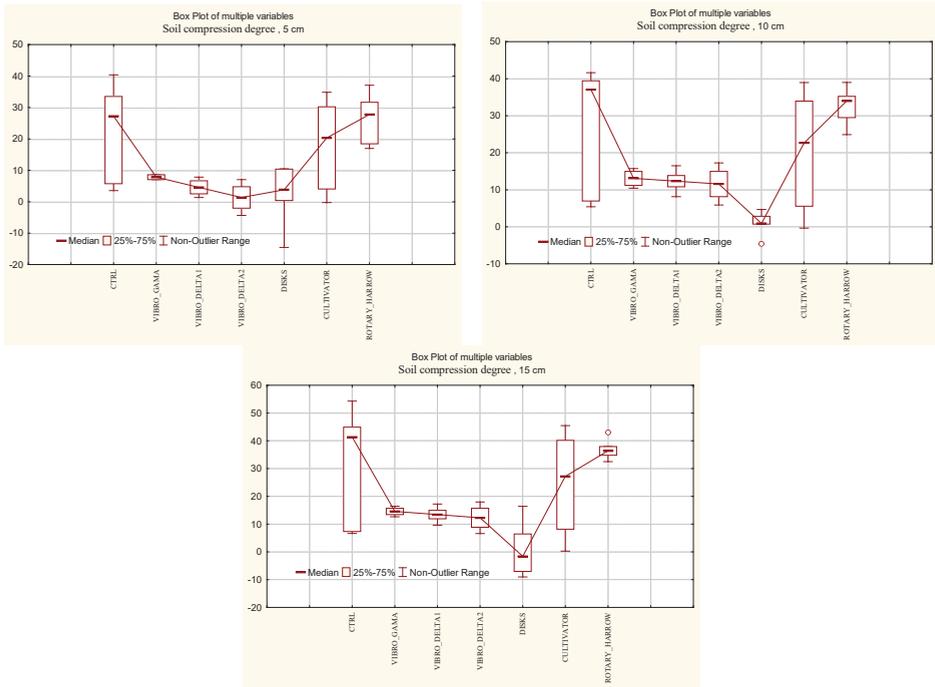


Figure 6. Variation of soil compression degree for seedbed preparation, for three depth

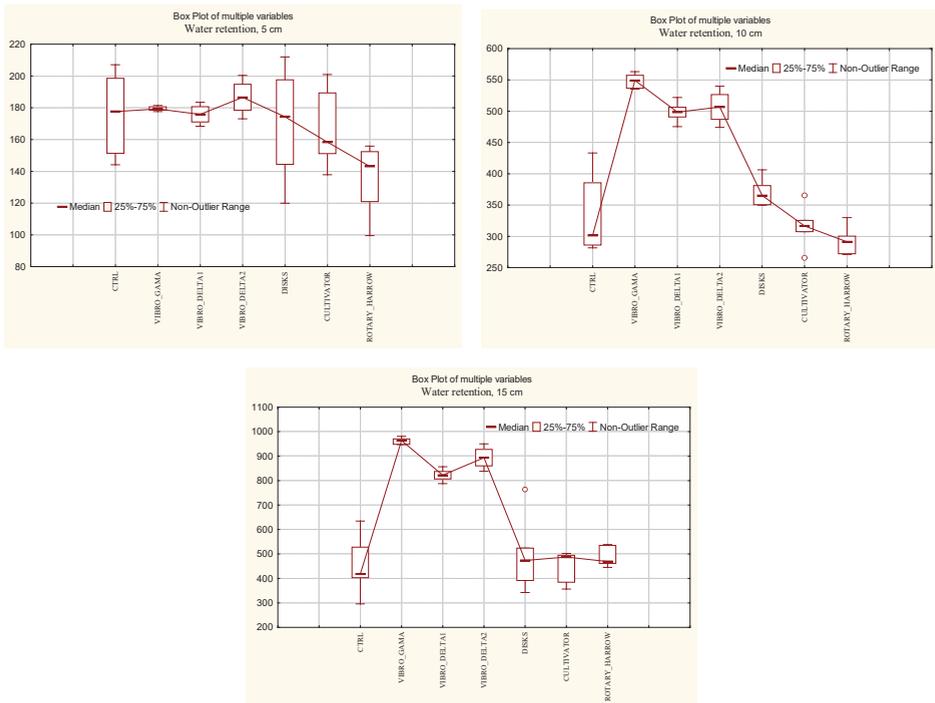


Figure 7. Variation of water retention for seedbed preparation, for three depth

## CONCLUSIONS

The process of soil compaction due to natural factors appears under the form of some genetic consolidated horizons. The situations which lead to the occurrence of the phenomenon of soil compaction are divided between the action of natural and anthropogenic factors.

During the action of the wheeling system of the tractors and the agricultural equipment on the soil, it is subjected to some mechanical efforts, which, through their action, make it shift laterally (refulation), vertically (compression) and horizontally (shear). The effect of the compression is transmitted in the layers of the soil in all directions, under the form of a pressure, and thus their propagation is insignificant at depths greater than 80 cm.

The physical characteristics like bulk density, total porosity and compression degree modify according to the soil works. The modification of these properties is hard to notice (except for the compression degree) during a year because the soil has the tendency, in normal conditions, to get back to the initial state and to estimate the negative effects which appeared after the impact produced by its processing with mechanical means.

Several researches show that in a long period of time, the evolution of the physical properties in a certain direction takes place at a slow rhythm, after a short period of time when they start to stabilise. This research attempted to emphasise the fact that the process of compaction plays a negative role on the physic-mechanical properties in the six methods for seedbed preparation.

The research investigated six methods for seedbed preparation in forestry nursery performances and the environmental impact of several active elements, at certain soil depths.

Following the six methods of seedbed preparation, it was found that the most efficient method, following the five physical-mechanical properties of the soil, is when using Vibro\_Delta2.

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## THE INFLUENCE OF THE AGRICULTURAL PRACTICES AND ENVIRONMENTAL CONDITIONS ON THE SOIL MICROBIAL COMMUNITY IN *Camelina sativa* CULTURE

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### Abstract

*Camelina sativa*, belonging to the Brassicaceae family, is a plant with high potential to be cultivated for biomass for biofuel production, including on less fertile soils. In Moara Domneasca farm (of UASMV Bucharest-Ilfov County) different camelina cultures were conducted using 'Mădălina' new variety. The aim of the study was to evaluate the microbial community in different stages of the camelina cultivation. The analysis targeted the level of the total aerobic bacteria, fungi, actinomycetes and lactic bacteria. The cultural practices have less affected the total bacterial content during an entire culture cycle, while the addition of initial nitrogen fertilisation decreased significantly (three logarithmic units) the level of the total bacteria, including the actinomycetes. No significant inhibition of the initial fertilisation was noticed in the case of fungi and lactic bacteria.

**Key words:** *Camelina sativa*, soil microbiology, fertilisation, fungi, actinomycetes, lactic bacteria.

### INTRODUCTION

Nowadays, environmental conditions and agricultural practices are very important, having an important influence on the soil microbial community.

*Camelina* (*Camelina sativa* L. Crantz) has the popular names gold of pleasure and false flax. It is an annual member of the Brassicaceae family (Putnam et al., 1995; Zubr., 2003) from south-eastern Europe and south-western Asian steppe region (Dobre et al., 2014).

It is a very good source of animal feed products and human food because *Camelina sativa* contains essential fatty acids, particularly n-3 (omega-3) fatty acids (Waraich et al., 2013; Belayneh et al., 2018). The oil obtained from camelina seeds has a high content of fatty acids with 50-60% unsaturated fatty acids, 35-40% omega 3-fatty acids and 15-20% omega 6-fatty acids. The main attractive features are: drought and frost tolerance, disease and pest resistance, a considerably high seed oil content (Belayneh et al., 2015).

Romania presents a considerable risk to climate change, its effects being clearly reflected by changes in temperature and precipitation,

mainly since 1961 and until now. The most affected areas, according to relevant international reports and analyzes climatological data series for the period 1901-2020 conducted by the National Meteorological Administration, being located in the southern, south-eastern and eastern part of the country. The main risks that Romania faces in the short and medium term consist in the significant increase of the average annual temperature, the decrease of precipitations and the general occurrence of extreme climatic events.

Severe pedological drought coupled with high water consumption between July and August lead to a reserve of water in the soil often below the wilting point on large agricultural areas.

Romania is characterised by a temperate continental climate and the precipitations fall in different forms. Most of the year there is liquid precipitation, especially in the hot season. In the cold season, especially in winter, the precipitations are in solid state, but there are also mixed precipitations (sleet, drizzle, etc.).

Soil biodiversity can have a major impact on the resistance and resilience of the ecosystem to climate change, which is particularly

relevant for the management of future crops. Current approaches to anticipating the effects of climate change in agriculture completely neglect underground biodiversity (Lewis et al., 2018).

Different groups of microorganisms have a varying degree of resistance to drought. Thus, bacteria and fungi are more sensitive, compared to actinomycetes, the low water content of the soil favouring both vegetative development and sporulation of the latter (Wardle et al., 1999).

Under arid conditions, factors such as the availability of nutrients, organic matter, soil texture and temperature are unfavourable to the development of soil microorganisms. Among the stressors, short-term drought could be a major stressor affecting the diversity and activity of bacterial populations, due to reduced substrate diffusion in dry soils and increased needs for C and N. In soils affected by drought an important role mesophilic bacteria also play thermophilic bacteria (Wardle et al., 1999).

Humidity has a high influence on soil microorganisms, excessive water levels decreasing both the frequency and activity of microorganisms, which is due to the reduction of the possibility of oxygen supply.

Excessive water levels in the soil decrease both the frequency and the activity of microorganisms, which is due to the reduced possibility of oxygen supply. The enzymatic activities in the soil are the result of the integration of complex synthesis processes, of the persistence, stabilization and catalytic regulation as well as of their location (intracellular or extracellular) inside or on the surface of the micro-aggregates. All these processes can be dynamically influenced by changes in the physical, chemical and biological composition of the soil (Sassenrath et al., 2018).

Modern, intensive, highly productive agriculture exerts significant demands on the soil and an insufficient knowledge of how the soil reacts to such increased demands can have negative consequences, manifested by processes of degradation, even destruction of its production capacity (Hamza et al., 2005). When approaching a certain type of tillage system, the soil, plant and climate conditions that can influence or can be influenced by that

system must be taken into account (Franzluebbers, 2002). The beneficial action of the tillage system on a crop factor must keep the other factors at an acceptable level, so that the increase of agricultural production, the decrease of fuel consumption or the increase of the soil production capacity can be possible through economic optimization solutions. Soil works, in addition to the unique and direct effects, beneficial in plant cultivation technologies, induce in the soil and lasting effects, which act on the physical and physical-mechanical, chemical and biological properties of the soil, modifying them (Canarache, 1991). Climate change predicts rainfall changes with increased annual rainfall in some regions and more intense rainfall events. Higher rainfall is often correlated with increased nutrient leaching (Austin and Vitousek, 1998), which exacerbates the risk of human and environmental health problems, as highlighted by the Millennium Ecosystem Assessment.

Therefore, it is crucial to reduce nutrient losses through leaching in order to preserve the environment and protect human health. A number of recent studies suggest that soil biota, including arbuscular mycorrhizal (AM) fungi, improves the nutrient cycle in agroecosystems and reduces leaching losses (Bender & Van der Heijden, 2015; Cavagnaro et al., 2015). However, it is not known whether the fungal capacity of AM to reduce nutrient losses is maintained in different precipitation scenarios. Good management of soil biota can mitigate the negative consequences of climate change and, in particular, of high rainfall. Previous studies have shown that mushrooms have an impact on nutrient leaching (Gounani et al., 2011).

## MATERIALS AND METHODS

In order to characterize the influence of the agricultural practices and environmental conditions on the soil microbial community in *Camelina sativa* culture our interest was focused on evaluating the level of the total aerobic bacteria, fungi, actinomycetes and lactic bacteria in the plots soil. The period analysed in this study was between November 2019 and October 2020.

### *Location characteristics*

The experiments were conducted in Moara Domnească farm, Călărași County. Moara Domnească farm belongs to Belciugatele experimental unit of the University of Agronomic Sciences and Veterinary Medicine of Bucharest. The type of soil is reddish preluvosoil, having loam-clay texture.

The extreme months from a thermal point of view are July and January. July is the warmest month of the year, with an average temperature of 24.25°C. January is the coldest month of the year, with the lowest temperature values, with an average temperature of 0.95°C.

The extreme months from a precipitation point of view are January and June. January is the poor month in precipitation of the year, with totally precipitation 1.60 mm. June is the richest month in precipitation of the year, with the highest precipitation values, with totally precipitation 131.40 mm.

The meteorological data of the location were collected by an automated meteo-station WatchDog 2900 ET and its Software Pro9, implanted in the farm.

### *Experimental plots and sampling*

The aim of the study was to evaluate the microbial community in different stages of the camelina cultivation. For the trials was used the new camelina variety, Madalina, patented in 2018 by the UASMV Bucharest. The employed technology for camelina was the following: double culture, on an initial surface of 2108 m<sup>2</sup>; the used tractor was U 683 DT. In June 2020, the first step was to straw grain harvester - rotary mower (1.2 m) then gathered plants with rotary rake and then voted with round ballot socket.

After that was prepared the land by milling - 1.5 m - one passage), fertilization (small 300) with ammonium nitrate - 100 kg (475 kg/ha), then sowing (SUP 21) - A1 - (3.2 kg/2108 m<sup>2</sup>) the Mădălina variety with a seeding rate of 10-12 kg/ha.

After two weeks the soil was irrigated with U683 DT + EEP 600 20 m<sup>3</sup>/ha because there wasn't precipitation. After another two weeks we observe that the weed begun to sprout and was applied Panther herbicide 0.5 l/ha. In September 2020 was started an autumn culture, by the use of herbicide with Glyphothim 3-4 l/ha (p = 5 atm., N = 300 l/ha).

The field was prepared as follows: destruction of vegetation with U683 DT + GD 3.4 and tilling the soil with U683 DT + PP 3X30. It was used a complex fertilizer to fertilize the soil with NP 20:20 with U683 DT + MIC-300 machine (Figure 1) in a dose of 250 kg/ha.



Figure 1. Fertilization of soil with U683 DT + MIC-300 machine

In the same day it was prepared the land with U683 DT + GD 3.4 machine and then we prepared the germination bed with U683 DT + C 3.9 machine. After that was sowed with U683 DT + SUP 21; gearbox diagram A2; B = 2.625 m the Mădălina variety (2018); G = 60%; N = 12 kg/ha. Later on, in October, a roller with U683 DT + TN 3 (Bl = 3 m) machine was used in the technology.

At different periods (before and after culture), we have collected 5 soil samples of 200 grams from different 4 corners of the field and one from the middle of it. In that 4 corners we have collected soil from one meter distance from the margins of the field. All the soil samples were collected from 20 centimetres deep, then put in sterile container and then transport in optimal conditions to the laboratory.

### *Physico-chemical analysis of the soil*

After that the analysis of all the soil samples were conducted in the agrochemistry laboratory of UASVMB. Simultaneously, the nutrients and agrochemical indices from the culture substrates from the experimental variants were analyzed. The analyses performed were pH in aqueous extract 1:2.5 and potentiometric dosing (SR ISO 10523-2012), soluble salt content extraction in water 1:5 and conductometric dosing (SR EN 27888-1997), N, P, K extraction in distilled water 1:5 and colorimetric dosing with AFDS for nitrates,

colorimetric dosing with Nessler reagent for  $\text{N-NH}_4^+$ ,  $\text{P-PO}_4^{3-}$  colorimetric dosing with Duval reagent and  $\text{K}^+$  flame-photometric dosing (STAS 7184/19-82).

The analysis methodology and the interpretation of the results were performed according to the existing standards and norms in our country (ICPA soil and plant analysis methodology, 1980).

#### Microbiological analysis

In the analysis were targeted the following microorganism groups: total aerobic bacteria, total fungi, actinomycetes and lactic acid bacteria. Specific cultivation media were used for each microbial group, respectively: Nutrient Agar, DRBC, Gause and MRS + Cycloheximide.

After preparing decimal dilutions from the soil samples, the suspension was spread in Petri Dishes, followed by the cultivation during 24-48 hours at 27°C. The developed colonies were counted and the CFU/ml was calculated accordingly.

## RESULTS AND DISCUSSIONS

In Romania, at Moara Domnească was an extremely dry period with 580.20 mm per year, according to November 2019 - October 2020 studies. Temperature influences almost every aspect, in special the activity of enzymes, according to Figure 2.

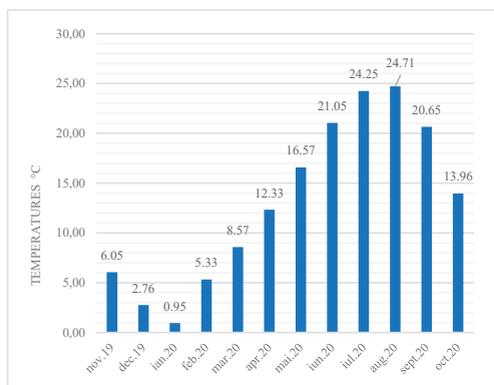


Figure 2. Average temperatures from Moara Domnească farm

Soil moisture is another abiotic factor that decisively influences soil respiration. In conditions of extreme drought, metabolic

activity of microorganisms become latency states. The intensity of precipitation had a strong positive effect on the amount of nutrients lost from the soil by leaching (Figure 3). The results on climatic parameters show a year with dry rainfall and air and soil temperatures that have exceeded normal values. In most months of the year, there was a precipitation deficit. The air temperature registered, in all the months of the year, values higher than the calculated multiannual normal. Influence of temperature, humidity and pH in the soil is very important.

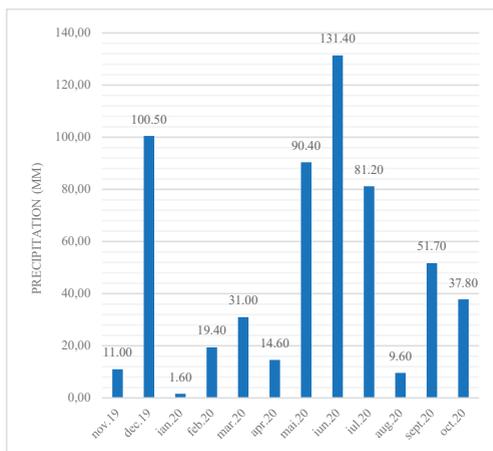


Figure 3. Monthly precipitation from Moara Domnească farm

According to Table 1, it was realized that there is a basic pH nearly to 8, a good quantity of hummus and a negligible amount of salts. The main inorganic forms of N in soils are ammonium ( $\text{NH}_4$ ) and nitrate ( $\text{NO}_3$ ), which are both useable by plants. Organic nitrogen may be present as a living organism, as humus, or as the intermediate products of organic matter decomposition. The level of ammonium in soil is between 4.25 and 8.50 ppm. The normal background level of nitrates in soil not fertilized or used for commercial crops ranges from 5 to 10 parts per 1 million (ppm), in our study was found the lowest value 2.25 ppm and the highest value 9.00 ppm. In conclusion, N is present in our soil in a good concentration. Agrochemical indices from the culture substrates from the experimental variants that we analysed are in a constant concentration.

Table 1. Soil analyses from Moara Domnească farm

Variant	pH	H, %	Soluble salts, %	ppm content						
				N-NH <sub>4</sub>	N-NO <sub>3</sub>	Σ N	P <sub>AL</sub>	K <sub>AL</sub>	Ca	Mg
1	7.9	1.320	0.011	4.25	2.25	6.50	1.95	69	20	11
2	7.9	1.322	0.058	6.40	4.50	10.90	2.06	70	18	12
3	7.9	1.340	0.084	4.25	9.00	13.25	2.10	70	15	12
4	7.6	1.128	0.026	8.50	9.00	17.50	2.98	56	21	10

The research aimed at determining if the *Camelina sativa* employed technology has the capacity to change soil microbial community. According to average temperatures and monthly precipitation collected from Moara Domnească farm, the results of the studies (Figure 4) describe the total aerobic bacteria, fungi, actinomycetes and lactic bacteria during different stages of camelina culture, respectively November 2019, June 2020, September 2020 and October 2020.

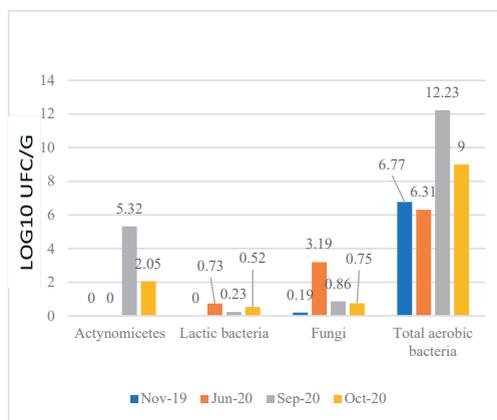


Figure 4. Microbial evolution of camelina stages in different periods

Actinomycetes level increased in September, after the first cycle of *Camelina sativa*, Mădălina to  $10^5$ CFU/g. They play major roles in the cycling of organic matter, has major contribution for soil and environment by nitrogen fixation (Absar et al., 2017). However, after the fertilisation, one month later, their level decreased to  $10^2$ CFU/g, due to an inhibitory mechanism which could not be explained. Lactic bacteria have less significant levels in soil after the first cycle, which was expected (10 CFU/g); however, their presence may demonstrated an increase of fertiliser solubility, very good at improving pore space

in soil ventilation, improve nutrient availability from compost or other organic material.

Very important is that lactic bacteria can directly promote plant growth (Higa et al., 2004).

Fungi are in a relatively low level in soil ( $10^1$ - $10^3$  CFU/g) because in most of case they decompose recalcitrant organic residues high in lignin and cellulose (Boer et al., 2010).

Regarding the influence of autumn plowing on different groups of microorganisms, it is found that microscopic fungi are more numerous, with the proliferation primarily of those of the genera *Fusarium* and *Aspergillus*. Of these, the largest share is *Aspergillus niger*. In spring soils, *Aspergillus ochraceus* and *Trichoderma viridis* predominate, as well as some fungi that are antagonistic to some pathogens which is in accordance to other authors (Abdel-Azeem et al., 2016).

Total aerobic bacteria increase in September 2020 and October 2020, after the first cycle of Mădălina culture because they grow in many different microenvironments and expand rapidly, reaching a level of  $10^{12}$  CFU/g. They could be more competitive when in soil are digestible simple sugars that are ready available in rhizosphere. Root exudates, dead plant debris, simple sugars, and complex polysaccharides are abundant in this region (Sylvia et al., 2005). During other studies in China (Hu et al., 2019) in similar conditions noticed that not exist significant inhibition of fungi and lactic bacteria.

## CONCLUSIONS

The cultural practices have less affected the total bacterial content during an entire culture cycle, while the addition of initial nitrogen fertilisation decreased significantly (three logarithmic units) the level of the total bacteria, including the actinomycetes. No significant

inhibition of the initial fertilisation was noticed in the case of fungi and lactic bacteria.

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## INFLUENCE OF BIOTIC AND ABIOTIC FACTORS ON MAIZE CROP YIELD IN TRANSYLVANIAN PLAIN CONDITIONS

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### Abstract

*The yield of a crop is dependent on the interaction of biotic and abiotic factors, the most important being the pedo-climatic conditions and the attack of diseases and pests. The aim of this paper is to evaluate the impact of the interaction of biotic and abiotic factors on the yield of the Turda 332 maize hybrid in the period 2018-2020. A multifactorial experiment was organized, with the following graduations: factor A - climatic conditions in the experimental years (2018-2019-2020), factor B - tillage system (conventional, minimum tillage with chisel, minimum tillage with disc, no-tillage), factor C - foliar fertilizers (different grades of mineral fertilization with macro and microelements). The obtained results indicate that the way of processing the soil has an influence on the water reserve in the soil and on the yield elements, but the biggest impact has the environmental conditions, in 2018 and 2020, favorable years for maize cultivation, the yield was 8971 kg/ha respectively 8578 kg/ha, and in 2019, a less favorable year, the yield was 5581 kg/ha. The frequency and intensity of the attack of diseases and pests are influenced by the variation of climatic conditions, in 2018 and 2020 registering the highest frequency of attack, the tillage system as well by the level of fertilization, in the variants with foliar fertilization registering the lowest attack.*

**Key words:** maize, yield, tillage systems, *Fusarium sp.*, *Ostrinia nubilalis*.

### INTRODUCTION

In the current climatic conditions, the identification of new technological solutions for the cultivation of maize, are important premises for increasing the stability of yield. The appearance and installation of temperatures of 32°C or above this threshold, determines the appearance of the heat phenomenon, a phenomenon that manifests itself quite frequently in the Transylvanian Plain. According to the data of the National Meteorological Agency (NMA), the evolution of heat intensity in Romania, in the period 1961-2010, highlights an upward trend, started in 1981, a trend that continues today (Sin and Popescu, 2015). Sandu and Mateescu (quoted by Sin and Popescu, 2015), states that for maize, according to climate predictions of rising air temperatures, there will be a shortening of the vegetation period by seven days until 2020 and 12 days until 2050 and decrease in yield with 14% by 2020 respectively 21% by 2050, depending on soil water deficits, especially in the grain filling phase (July-August).

From sowing to harvesting maize is subject to many stressors, both biotic and abiotic, these factors together make up an ecosystem.

Crop yields depends by many environmental factors (Ranjan et al., 2006; Espósito et al., 2009) such as temperature, precipitations, tillage or soil type and moisture and whose effects are difficult to predict (Marin et al., 2012; Rusu, 2014).

The introduction of conservative agriculture systems seeks to improve, conserve and make more efficient use of natural, biological and water resources (Guş et al., 2003; Rusu et al., 2009).

Soil conservation systems have specific characteristics in different areas and countries, related to the specifics of ecology and cultivated plants, thus requiring different implementation models (Florea, 2003; Guş et al., 2004; Feiza et al., 2005; Jităreanu et al., 2006; Rusu et al., 2009; Moraru and Rusu, 2010).

The yielding of plants cultivated in no-tillage systems is, generally, lower than of plants from conventional tillage systems (Woźniak, 2013).

In addition to the genetic factor, the cultivation technologies adapted to the zonal ecological conditions, can make an important contribution in limiting the yield losses, from one year to another.

At the same time, it is known that technological elements can influence in one way or another the evolution of biotic factors in an agroecosystem, factors that can manifest under certain conditions a significant impact on maize yield. Also, climate change and extreme phenomena increase the vulnerability of plants to the attack of pests and pathogens.

Biotic factors also have a high impact on the formation of yield, as they are the living parts of an environment, such as plants, animals and microorganisms, especially specific diseases and pests.

Maize infection with *Fusarium* sp. it can occur throughout its cultivation. This can cause different types of diseases in the vegetative and generative organs of the plant. Along with these infections, mycotoxins are often produced and accumulated in the affected tissues, which could pose a significant risk to human and animal health upon entering the food and feed chain (Oldenburg et al., 2017).

The rot of cobs is the most common disease of maize, caused by several species of the genus *Fusarium*. The symptoms are those of mold, white to pink or salmon, which appears on isolated caryopsis, scattered or grouped on several caryopsis (CPN, 2021).

The penetration of agents into the plant is more frequent through wounds, produced by insects, such as corn borer, poultry or hail (Oldenburg et al., 2017).

Factors contributing to *Fusarium* spp. Infections are climatic/meteorological conditions, plant cultivation technology/protection measures and stress conditions. Agricultural practices that allow effective management to reduce the risk of *Fusarium* infection and the accumulation of mycotoxins are: tillage that ensures rapid decomposition of plant residues infected and contaminated with *Fusarium*, selection of hybrids with low sensitivity, extensive crop rotation, a balanced fertilization and insecticide treatment alone or in combination with fungicides, where appropriate (Oldenburg et al., 2017).

The corn borer (*Ostrinia nubilalis* Hbn.) is the main pest of maize cultivation in areas of Transylvania, one of its favorable areas (Barbulescu et al., 2001; Georgescu et al., 2015), which in certain climatic conditions and non-compliance agrophytotechnical measures can cause significant damage. Due to the climatic conditions of May-June-July, which have changed in recent years and no longer fit the pattern of recent decades, the corn borer attack can be carried out with a different intensity from one year to another, so maize hybrids, tolerant to pest attack, may become sensitive in favorable years (Ivaş et al., 2013).

All the attack parameters of the corn borer ultimately lead to significant crop losses (Tărău et al., 2019). The application of chemical treatments against this pest could negatively influence the quality of yield. Thus, efforts to find alternative methods of limiting the harmful effects of diseases and pests are an important link in agricultural practices. One such method is the diversified use of mineral fertilization (Bocianowski et al., 2016).

Given these, the paper aims to investigate the influence of technological elements (soil tillage and fertilization system) on maize yield and the evolution of diseases and pests specific to this crop in the Transylvanian Plain, in 3 years of experimentation (2018-2020).

## MATERIALS AND METHODS

The researches took place in the period 2018-2020, at the Agricultural Research and Development Station Turda (ARDS Turda), on a Faeoziom type soil.

The experience is a three-factor one, and the surface of an experimental plot is 48 m<sup>2</sup>. During the experiment, the sowing of maize was done with the machine MT6-Maschio Gaspardo. The sowing density was 65000 plants/ha and the seed incorporation depth was 5 cm. The preplant was winter wheat. The biological material was represented by the maize hybrid Turda 332, created at ARDS Turda.

To meet the proposed objectives, a multifactorial experiment was organized, with the following graduations: factor A-climatic conditions in the experimental years: a<sub>1</sub>-2018; a<sub>2</sub>-2019; a<sub>3</sub>-2020; factor B-tillage system: b<sub>1</sub>-conventional tillage system (plowing with

turning the furrow), b<sub>2</sub>-minimum tillage system (with chisel), b<sub>3</sub>-minimum tillage system (with disk harrow), b<sub>4</sub>-no-tillage system; factor C-foliar fertilizers: c<sub>1</sub>-mineral fertilization (control); c<sub>2</sub>-mineral fertilization + Haifa 19:19:19 + Mg + Microelements (5 kg/ha); c<sub>3</sub>-mineral fertilization + Folimax Oleo 12-04-24 + 2.0% MgO + 36.5% SO<sub>3</sub> + Microelements (1.5 kg/ha), c<sub>4</sub>-mineral fertilization + Folimax Gold 27.0% N + 1.5% MgO + 0.02% B + 0.2% Cu + 0.02% Fe + 1.0% Mn + 0.02% Mo + 0.02% Zn (3 l/ha). Two foliar treatments were performed, the first treatment was performed in the 8-10 leaf phenophase, and the second treatment was performed in the 10-12 leaf phenophase.

Mineral fertilization was carried out in two phases: simultaneously with sowing, applying a complex fertilizer type NPK (27%N:13.5%P<sub>2</sub>O<sub>5</sub>:0) at a dose of 250 kg/ha, and on vegetation in the phenophase of 6-8 leaves administering a CAN type fertilizer (27% N: 7% CaO: 5% MgO) in a dose of 120 kg/ha, in all variants.

The climatic conditions in the three experimental years were different, with temperature variations from one month to another even from one decade to another, often registering temperatures higher than normal (Table 1).

From a thermal point of view, the 3 years studied were characterized as warm years, with an increase in the average annual temperature,

compared to the average of 60 years during the vegetation period of 2.7°C (2018), of 1.6°C (2019) and 1.0°C (2020).

During the vegetation period of maize crops, the average monthly temperatures increased compared to the multiannual average, except for May (in 2019 and 2020) which had a cool character with negative deviations from the average of 1.4°C, respectively 1.3°C.

Regarding the amount of precipitation from April to October, a variation can be observed from one month to another, which causes the maize to be affected by the transitions from one extreme to another, meaning from significant amounts of precipitation to days with prolonged drought.

The monthly precipitations registered in 2018 and 2020 had negative deviations from the normal in April and May, and in 2019 in the period June-July the lowest values were registered from a quantitative point of view (Table 2).

Although they were oscillating, still the temperatures recorded in the vegetation period correlated with the precipitations fallen in the same period met favorable conditions for the optimal development of the maize crop in 2018 and 2020, in 2019 the lack of precipitation was felt by the plants during the most important, from the second decade of August until the physiological maturity of the plants.

Table 1. The average air temperature (°C) during the period 2018-2020, ARDS Turda

Experimental year	Average air temperature (°C)						
	Moon/Decade	April	May	June	July	August	September
2018	Decade I	12.4	19.6	20.5	18.5	23.2	19.6
	Decade II	16.6	15.6	20.5	20.5	22.3	18.8
	Decade III	17.0	20.7	17.5	22.0	21.6	11.5
	Monthly average	15.3	18.7	19.4	20.4	22.3	16.7
	Average 60 years	9.9	15.0	17.9	19.7	19.3	15.1
	Deviation	5.4	3.7	1.5	0.7	3.0	1.6
2019	Decade I	11.9	10.8	19.7	21.2	20.6	20.4
	Decade II	9.5	14.6	23.6	17.4	22.2	16.5
	Decade III	12.6	15.2	22.1	22.4	23.5	14.4
	Monthly average	11.3	13.6	21.8	20.4	22.1	17.1
	Average 60 years	9.9	15	17.9	19.7	19.3	15.1
	Deviation	1.4	-1.4	3.9	0.7	2.8	2
2020	Decade I	8.7	12.1	17.4	20.9	22.9	19.1
	Decade II	11	16.5	19.2	18.4	20.6	19.3
	Decade III	11.1	12.5	20.8	21.1	21	15.1
	Monthly average	10.3	13.7	19.1	20.2	21.5	17.8
	Average 60 years	9.9	15	17.9	19.7	19.3	15.1
	Deviation	0.4	-1.3	1.2	0.5	2.2	2.7

Table 2. The amount of precipitation (mm) during the period 2018-2020, ARDS Turda

Experiemntal year	Precipitation (mm)						
	Moon/Decade	April	May	June	July	August	September
2018	Decade I	5.4	16.8	13.8	51.9	20.6	15.2
	Decade II	14.4	33.4	67.5	28.2	0.0	10.0
	Decade III	6.4	6.6	17.0	5.6	17.6	4.6
	Monthly amount	26.2	56.8	98.3	85.7	38.2	29.8
	Average 60 years	45.9	68.7	84.8	77.1	56.5	42.5
	Deviation	-19.7	-11.9	13.5	8.6	-18.4	-12.7
2019	Decade I	3.8	34.8	30.6	7.6	59.6	0
	Decade II	34.8	38.8	5.6	25.2	3	0.4
	Decade III	24	78.8	32.6	2.2	1.2	19
	Monthly amount	62.6	152.4	68.8	35	63.8	19.4
	Average 60 years	45.9	68.7	84.8	77.1	56.5	42.5
	Deviation	16.7	83.7	-16	-42.1	7.2	-23.1
2020	Decade I	0	10.2	16	23	3.6	6.6
	Decade II	0.8	11.2	115	51.6	53.6	0
	Decade III	17	23	35.6	12.2	0.8	50.8
	Monthly amount	17.8	44.4	166.6	86.8	58	57.4
	Average 60 years	45.9	68.7	84.8	77.1	56.5	42.5
	Deviation	-28.1	-24.3	81.8	9.7	1.5	14.9

## RESULTS AND DISCUSSIONS

In addition to the amount of rainfall during the vegetation period and the water reserve in the soil is a decisive factor in achieving production. Monitoring the water supply in the soil, even before sowing the crop, is very important because all stages of development are dependent on rainwater and soil, taking into account the fact that in the research perimeter it is the only source of water.

According to data from the literature (Guş et al., 2004; Feiza et al., 2005; Jităreanu et al., 2006; Rusu et al., 2009; Moraru and Rusu, 2010) by applying conservative tillage systems reserve of water in the soil registers an increase compared to the conventional tillage system, this fact is also confirmed by the data recorded in this experiment, where it can be seen that both on the depth of 0-20 cm (Figure 1) and on the depth of 0-50 (Figure 2) the water reserve is higher in most conservative tillage variants.

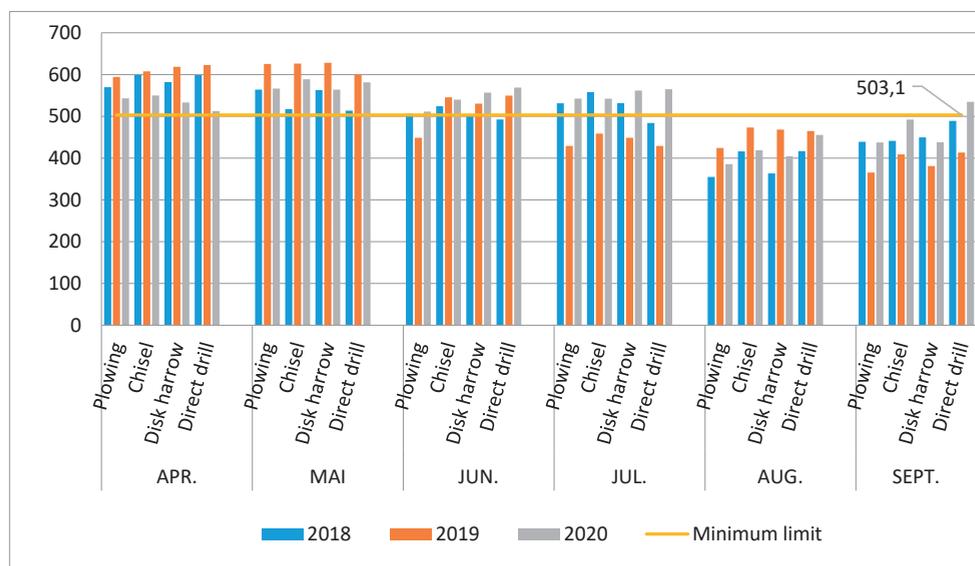


Figure 1. Momentary soil moisture reserve on the layer 0-20 cm (m³/ha)

In 2018, the soil water reserve exceeded the minimum limit, on both layers, during the sowing period and until the beginning of August, when there were decreases in the values below the minimum limit level, the maize being in that period in the phenophase baking in milk and staying below this limit until harvest.

The year 2019 was quite similar to 2018 in terms of soil water supply, in the early stages of development, but since July the values have started to fall below the minimum limit.

Maize cultivation has been affected by the lack of water since the split phenophase to harvested, the most visible effect being grain

ripening, which implicitly leads to decreased yield.

And in the third experimental year the soil water supply was at appropriate values for a good development of the crop, until the end of August, when the corn was in the milk-wax phenophase, when the first decreases in water supply. These low values remained until the end of September.

The lack of precipitation correlated with the high temperatures means that the yield obtained from the maize crop are negatively affected, and the plants consume more energy in order to obtain significant yield increases, the climatic conditions being still the most important factor determining the yield of a cultures.

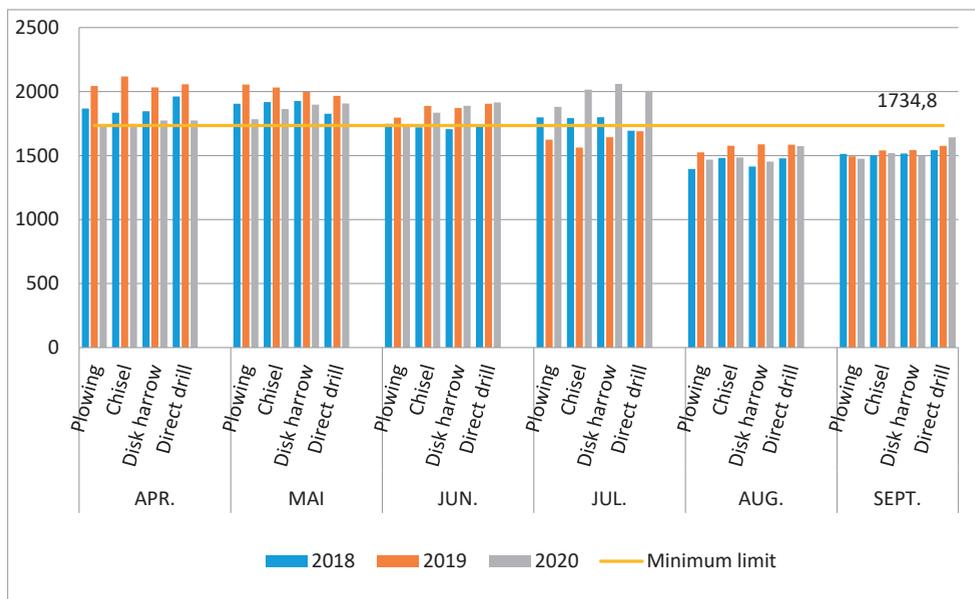


Figure 2. Momentary soil moisture reserve on the layer 0-50 cm (m³/ha)

As can be seen in Table 3, the yield recorded in the three years studied varied, the years 2018 and 2020 having more favorable conditions for culture and 2019 being less favorable.

The lack of precipitation during the ripematurity period left its mark on the yield achieved in 2019, registering the smallest increase in yield, of only 5581 kg/ha, with a very significant negative difference of 2129 kg/ha compared to the average of those 3 years. Although the precipitation regime in the milk-baking phenophase was deficient in 2018 and 2020, the crop still managed to overcome this

period and record significant increases in yield, with very significant positive differences of 1261 kg/ha (2018) respectively 868 kg/ha (2020) compared to the average of the 3 years.

The application of conservative tillage systems, from the point of view of the obtained yields is not efficient, in all the 3 variants of conservative tillage registering very significant decreases of yield from 603 kg/ha in case of tillage with disc harrow and up to 1780 kg/ha in the case of direct sowing.

Although conservation systems are seen as a way to protect and preserve the soil and its pro-

properties, in some cases the benefits of conservation systems are not visible in the short term. In addition to the basic fertilization applied simultaneously with sowing, the maize crop reacts positively to the application of foliar fertilizers, registering very significant yield increases of up to 611 kg/ha when applying the Folimax Gold product at a dose of 3 l/ha.

The tillage system influences the obtained yield, so that in the classical system an average production of over 8000 kg/ha was obtained in all fertilization variants, the lowest yields being registered in the no tillage system with differences of over 1000 kg/ha, in all fertilization variants. The application of foliar fertilizers can bring an increase in yield of up to 766 kg/ha when applying the Haifa product (Table 4).

Table 3. The influence of experimental factors on maize yield, Turda 2018-2020

Factor	Yield (kg/ha)	%	Difference
Experimental years			
Years average (control variant)	7710	100	0.00
2018	8971***	116	1261
2019	5581 <sup>000</sup>	72	-2129
2020	8578***	111	868
LSD (p 5%) 118; LSD (p 1%) 157; LSD (p 0.1%) 204			
Tillage systems			
Conventional (control variant)	8480	100	0.00
Minimum tillage (chisel variant)	7789 <sup>000</sup>	91	-691
Minimum tillage (disk harrow variant)	7871 <sup>000</sup>	93	-609
No-tillage	6700 <sup>000</sup>	79	-1780
LSD (p 5%) 119; LSD (p 1%) 180; LSD (p 0.1%) 290			
Foliar fertilization			
Basic fertilization (control variant)	7342	100	0.00
Basic fertilization + Haifa	7887***	107	545
Basic fertilization + Folimax Oleo	7658***	104	317
Basic fertilization + Folimax Gold	7953***	108	611
LSD (p 5%) 141; LSD (p 1%) 191; LSD (p 0.1%) 256			

The 3 experimental years were different, the climatic factor having a great influence on the yield. The years 2018 and 2019 were close in terms of yield values, the highest yield was

recorded in 2018 in the classical tillage system 9565 kg/ha, and the lowest yield was recorded in 2019 in the no tillage system of 3986 kg/ha (Table 5).

Table 4. The influence of the interaction of tillage system and foliar fertilization factors on maize yield, Turda 2018-2020

Variant	Yield(kg/ha)	%	Difference
Conventional x Basic fertilization (control variant)	8011	100	0.00
Minimum tillage (chisel variant) x Basic fertilization	7315 <sup>000</sup>	91	-696
Minimum tillage (disk harrowvariant) x Basic fertilization	7499 <sup>000</sup>	94	-512
No-tillage x Basic fertilization	6543 <sup>000</sup>	82	-1467
Conventional x Basic fertilization Haifa (control variant)	8777	100	0.00
Minimum tillage (chisel variant)x Basic fertilization + Haifa	7998 <sup>000</sup>	91	-779
Minimum tillage (disk harrow variant) x Basic fertilization + Haifa	8028 <sup>000</sup>	92	-750
No-tillage x Basic fertilization + Haifa	6744 <sup>000</sup>	77	-2034
Conventional x Basic fertilization + Folimax Oleo (control variant)	8365	100	0.00
Minimum tillage (chisel variant) x Basic fertilization + Folimax Oleo	7870 <sup>000</sup>	94	-495
Minimum tillage (disk harrow variant) x Basic fertilization + Folimax Oleo	7747 <sup>000</sup>	93	-619
No-tillage x Basic fertilization + Folimax Oleo	6651 <sup>000</sup>	80	-1714
Conventional x Basic fertilization + Folimax Gold (control variant)	8767	100	0.00
Minimum tillage (chisel variant) x Basic fertilization + Folimax Gold	7973 <sup>000</sup>	91	-793
Minimum tillage (disk harrow variant)x Basic fertilization + Folimax Gold	8210 <sup>000</sup>	94	-557
No-tillage x Basic fertilization + Folimax Gold	6861 <sup>000</sup>	78	-1906
LSD (p 5%) 270; LSD (p 1%) 375; LSD (p 0.1%) 520			

Table 5. The influence of the interaction between the soil tillage and the experimental years factors on maize yield, Turda 2018-2020

Variant	Yield(kg/ha)	%	Difference
Conventional x 2018(control variant)	9565	100	0.00
Minimum tillage (chisel variant) x 2018	9472	99	-94
Minimum tillage (disk harrow variant) x 2018	9473	99	-92
No-tillage x 2018	7374 <sup>000</sup>	77	-2191
Conventional x 2019 (control variant)	6553	100	0.00
Minimum tillage (chisel variant) x 2019	5865 <sup>000</sup>	90	-687
Minimum tillage (disk harrow variant) x 2019	5920 <sup>000</sup>	90	-632
No-tillage x 2019	3986 <sup>000</sup>	61	-2566
Conventional x 2020 (control variant)	9323	100	0.00
Minimum tillage (chisel variant) x 2020	8031 <sup>000</sup>	86	-1292
Minimum tillage (disk harrow variant) x 2020	8219 <sup>000</sup>	88	-1103
No-tillage x 2020	8739 <sup>000</sup>	94	-583
LSD (p 5%) 226; LSD (p 1%) 310; LSD (p 0.1%) 428			

### Results on cob fusarium wilt.

From the data presented in Table 6 we can observe that the frequency and intensity of the attack of *Fusarium* sp. were influenced differently by climatic conditions. The highest value of the attack frequency was recorded in 2018 (48.63). Compared to this, in 2019 and 2020 the frequency of the attack was reduced, with statistically assured differences compared to the control. The climatic conditions in 2018 were favorable for the fusariosis attack, this year the highest value was registered (5.55). Against the background of the climatic conditions from 2020, the frequency of the attack of *Fusarium* sp. was the lowest, with a significantly negative difference from the control variant.

The tillage system can influence the attack of pathogens. Data from the literature specify that by tillage are incorporated plant residues that may contain resistance spores of the pathogen. The data presented in Table 7 confirm the data in the literature, the frequency of the attack of fusariosis has the lowest value if the plot was shown and plant debris incorporated into the soil. In the case of the intensity of the attack, the highest value was recorded in the case of the undeveloped plot (5.06) followed by the plot in which a disc passage was made (4.91). Even if the plot

shows that the intensity was slightly higher, the low value of the degree of attack is given by the product between frequency and intensity.

The data presented in Table 7 confirm the data in the literature, the frequency of the attack of fusariosis has the lowest value if the plot was shown and plant debris incorporated into the soil. In the case of the intensity of the attack, the highest value was recorded in the case of the undeveloped plot (5.06) followed by the plot in which a disc passage was made (4.91). Even if the plot shows that the intensity was slightly higher, the low value of the degree of attack is given by the product between frequency and intensity.

To prevent the attacks of phytopathogens, a balanced fertilization is recommended, respecting the specific doses of each plant. A properly "fed" plant fights better against pests.

Additional fertilization can negatively or positively influence the attack of phytopathogens. The mean frequency of fusariosis attack was higher in the case of fertilization with Haifa and lower in the case of fertilization with Folimax Oleo and Folimax Gold, but the differences from the control were not statistically assured (Table 8). The intensity of the attack had the highest value (5.01) in the variant where no additional fertilization was done (Table 8).

Table 6. The influence of climatic conditions on the *Fusarium* attack, Turda 2018-2020

Year	Frequency (arcsin√%)	Difference compared to control %	Difference compared to control	Intensity (arcsin√%)	Difference compared to control %	Difference compared to control
2018	48.63	100	0.00	5.55	100	0.00
2019	33.47	68.8	-15.17 <sup>00</sup>	4.61	83.0	-0.95
2020	39.47	81.1	-9.17 <sup>0</sup>	4.48	80.8	-1.07 <sup>0</sup>
LSD (p 5%)			6.02			0.95
LSD (p 1%)			9.96			1.58
LSD (p 0.1%)			18.64			2.95

Table 7. The influence of tillage systems on the *Fusarium* attack, Turda 2018-2020

Variant	Frequency (arcsin√%)	Difference compared to control %	Difference compared to control	Intensity (arcsin√%)	Difference compared to control %	Difference compared to control
Conventional (control variant)	39.79	100	0.00	4.83	100	0.00
Minimum tillage (chisel)	40.38	101.5	0.60	4.72	97.8	-0.11
Minimum tillage (disk harrow)	41.61	104.6	1.82	4.91	100.6	0.08
No-tillage	40.32	101.3	0.53	5.06	103.8	0.23
LSD (p 5%)			2.14			0.41
LSD (p 1%)			2.98			0.57
LSD (p 0.1%)			4.00			0.77

Table 8. The influence of foliar fertilization on the *Fusarium* attack, Turda 2018-2020

Variant	Frequency (arcsin√%)	Difference compared to control %	Difference compared to control	Intensity (arcsin√%)	Difference compared to control %	Difference compared to control
Mineral fertilization (control variant)	41.36	100	0.00	5.01	100	0.00
Mineral fertilization + Haifa	42.75	103.4	1.39	4.70	97.7	-0.31
Mineral fertilization + Folimax Oleo	38.69	93.6	-2.67	4.96	98.9	-0.05
Mineral fertilization + Folimax Gold	39.30	95.0	-2.06	4.85	96.8	-0.16
LSD (p 5%)			2.93			0.39
LSD (p 1%)			3.90			0.52
LSD (p 0.1%)			5.05			0.68

### Results of *Ostrinia nubilalis* Hbn. attack.

Little information is available in the literature on the effect of fertilization on corn borer damage. Previous studies (Kolmanič, 2017; Bocianowski et al., 2016) report increases in the percentage of cobs attacked with supplemental doses of mineral nitrogen (Slovenia) or insignificant differences between fertilized variants with mineral fertilizer (Poland).

The results obtained in this paper are similar to those in Poland, even in the case of the interaction between the tillage system and fertilization, the corn borer attack being insignificant. The highest percentage of attacked cobs was registered in the foliar fertilized variants with the commercial product Haifa, while the lowest percentage appeared in the variants fertilized with the Folimax Gold product (Figure 3).

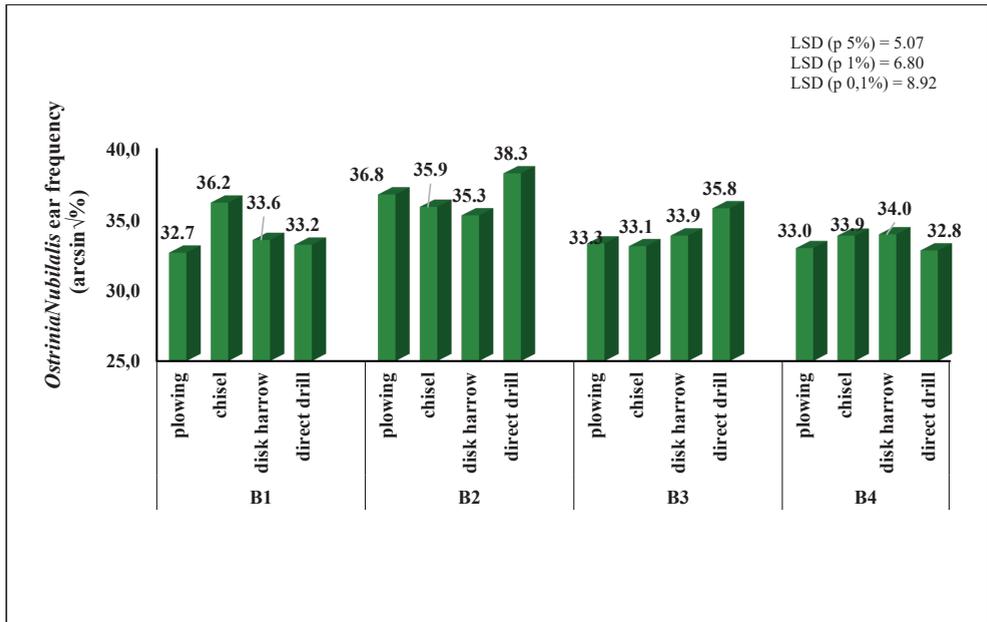


Figure 3. The influence of the interaction between the tillage system and fertilization on the frequency of the corn borer (*Ostrinia nubilalis* Hbn.) attack on cobs (ARDS Turda, 2018-2020)

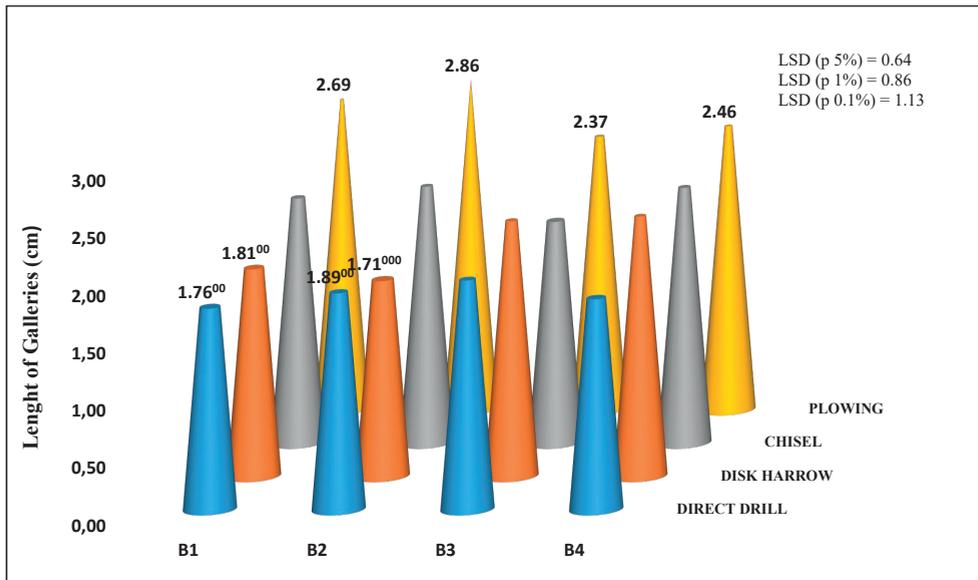


Figure 4. The influence of the interaction between the tillage system and fertilization on the length of the galleries produced by the corn borer (*Ostrinia nubilalis* Hbn.) (ARDS Turda, 2018-2020)

Regarding the intensity of the corn borer attack, represented by the length of the galleries (Bažok et al., 2009), (Figure 4), all interactions between systems and fertilization to some extent limit the corn borer attack to the control

(Conventional tillage x fertilization). The highest values of the length of the galleries are registered in the control variant fertilized with the Haifa product. Due to this fact, the variants fertilized with this product had the highest

statistically significant meanings as negative (Minimum tillage -disk variant x Haifa) (Figure 4).

Phelan et al. (1995) showed that sowing maize in the conventional system (plowing) did not influence the massive egg laying of *Ostrinia nubilalis*, but increased its probability, depending on the fertilizer used.

These results suggest that soil management practices and mineral fertilization may differently affect the attack of the corn borer, significantly in the case of the stem attack, represented by the length of the galleries, and insignificantly in the case of the cob attack.

## CONCLUSIONS

The lack of precipitation during the ripeness period is an important factor that leads to the decrease of the realized yield, in 2019 registering the smallest increase of yield, of only 5581 kg/ha, with a very significant negative difference compared to the 3 years.

The low amount of precipitation correlated with the high temperatures makes the plants consume more energy in order to obtain significant yield increases, the climatic conditions being still the most important factor that determines the yield of a crop.

Plowing with the return of the furrow is still the best agrotechnical method by which the attack of diseases and pests can be reduced, by tillage being incorporated the vegetal remains that can contain spores of resistance of the pathogen.

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## ENZYMATIC ACTIVITY OF TYPICAL CHERNOZEMS UNDER THE CONDITIONS OF THE ORGANIC FARMING SYSTEMS

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### *Abstract*

*Soil enzymes play a major environmental function by participating in biochemical processes related to the conversion of substances and energy into soil. The purpose of the research is to study the biological processes occurring in the soil under the influence of different farming systems. The activity of the following soil enzymes was studied: catalase, urease, dehydrogenase, protease and cellulase. The largest changes in the activity of soil enzymes were observed in the layer of 0-10 cm. Organic farming enhances the enzymatic activity at the level at which is found in uncultivated soil (fallow). The use of green manure increases the activity of most soil enzymes. The intensive farming system is characterized by a much lower enzyme activity in the 0-10 cm layer, as well as an increase enzyme activity in in the 20-30 cm layer, which is related to the soil tillage features (layer turnover). Hence the enzymatic activity directly depends on the farming system.*

**Key words:** enzyme, chernozem, farming system, fallow.

### INTRODUCTION

It is known that the enzymatic activity of the soil is associated with the activity of fungi, microorganisms, algae, protozoa and root secretions of plants. The highest potential activity of enzymes is in the upper part of the humus-accumulative horizon, because it is here that the largest reserves of organic matter and microbial biomass are concentrated. Thus, enzymatic activity reflects those changes in soil evolution that are caused by plowing, so it can characterize the biological activity of the soil to the greatest extent (Shi, 2011; Bautista-Cruz, 2015; Zhang, 2020).

One of the most stable indicators of soil biological activity is its enzymatic activity. Soil enzymes perform the most important ecological function, participating in biogeochemical processes related to the conversion of minerals, organic matter and energy.

Many years of studies have proven the effectiveness of diagnosing soil cover by biochemical methods. Low experimental error and high sensitivity to external factors contribute to the use of soil enzymatic activity as a diagnostic indicator of soil fertility and ecological status (Sinigani, 2013; Coleman, 2018; Zimmerman, 2011).

The aim of the study was to determine the impact of different farming systems (organic and intensive) on the change in the enzymatic activity of chernozem soils.

### MATERIALS AND METHODS

Typical deep medium loamy chernozems on the loess (molik, mollisol) located on the territory of Zinkiv district of Poltava region were selected for research. (forest-steppe zone, Ukraine). The soils are located on the plateau of the watershed between the rivers Psel and Vorskla. The area is wide undulating plain densely permeated with ravine-beam systems.

The selection of individual soil samples took place during 2018-2020 in the fields of farms operating under two radically different systems of agriculture. Organic technology farms abandoned plowing in 1975, herbicides and other agrochemicals in 1978, and mineral fertilizers a few years later. In the fields of the enterprise, working on traditional technology, a system of different tillage is used: deep loosening, plowing, disking and cultivation. The technology of growing crops involves the use of seeds, fertilizers and plant protection products only from the best domestic and foreign producers. New agricultural machinery

is used in the fields of the farm and elements of precision agriculture are introduced into production: GPS-monitoring systems, autopiloting, remote sensing methods, yield monitoring, variable sowing rates and differentiated fertilizer application.

The first soil profile is located on field with an area of 143 hectares, where in the crop rotation link a vetch yara (*Vicia sativa* L.) is grown for green manure – is a variant of the organic farming system (green manure). The crop rotation is shown in Table 1, and technological operations in Table 2.

Table 1. Crop rotation and fertilizer system during the research period

Variant/year	2018	2019	2020
Organic farming system (green manure)	vetch yara on green manure	winter wheat (green manure, 15 t/ha of green mass)	wintering peas - moved corn to silage
Fallow	weeds		
Organic farming system (compost)	corn for grain (20 t/ha of compost from cattle manure)	oat	soybeans
Intensive farming system	corn for grain (N130P30K30)	sunflower (N35P15K30)	corn for grain (N130P30K30)

Table 2. Technological operations for the period 2018-2020, variant organic farming system (green manure)

2018	2019	2020
- harvest of the predecessor in 2017 - earnings of crop residues by a disk cultivator to a depth of 6-8 cm - disking (12-14 cm) - early spring cultivation (4 cm) - pre-sowing cultivation (4 cm) - sowing of vetch yara (continuous sowing) - disking of green manure to a depth (6-8 cm) in two tracks - pre-sowing cultivation (5 cm) - sowing of winter wheat	- early spring harrowing - harvesting by direct combining - harvesting of straw - earnings of crop residues by a disk cultivator (10-12 cm) - cultivation (6-8 cm) - pre-sowing cultivation (5 cm) - sowing of winter peas	- cultivation (6-8 cm) - earnings of crop residues by a disk cultivator to a depth of 12-14 cm - pre-sowing cultivation (6 cm) - sowing corn for silage because the peas are gone - two inter-row cultivation - collection of green mass on a silo - cultivation (12-14 cm)

The second soil profile is located on a plot that has not been cultivated for over 30 years – it is variant fallow. Grow in the field association of legumes, grasses and cereals. The variant of fallow is control.

The third soil profile is located on field with an area of 94 hectares, where the compost is applied at a dose of 20 t/ha – is a variant of the

organic farming system. The crop rotation is shown in Table 1, and technological operations in Table 3.

Table 3. Technological operations for the period 2018-2020, variant organic farming system (compost)

2018	2019	2020
- harvest of the predecessor in 2017 - earnings of crop residues by a disk cultivator (6-8 cm) - disking (12-14 cm) - rolling by heavy ring-spur rollers (spring) - export and application of humus (compost) - cultivation to a depth of 6-8 cm - pre-sowing cultivation (6 cm) - sowing of corn - harrowing of ladders - three inter-row cultivation, and the last with hilling - harvesting - earnings of crop residues by a disk cultivator (6-8 cm) - cultivation to a depth of 12-14 cm	- spring provocative cultivation (3-4 cm) - pre-sowing cultivation (4 cm) - sowing of oats - post-emergence harrowing - harvesting by separate method - harvesting of straw - earnings of crop residues by a disk cultivator (10-12 cm)	- early spring harrowing - pre-sowing cultivation (4 cm) - soybean sowing - pre-sowing cultivation (4 cm) - new soybean sowing - post-emergence harrowing - three inter-row cultivation - harvesting by direct combining - earnings of crop residues by a disk cultivator (12-14 cm) - deep loosening to a depth of 26 cm

The fourth soil profile is located on field with an area of 125 hectares, where they use the full range of plant protection products and fertilizers. The crop rotation is shown in Table 1, and technological operations in Table 4.

The activity of the following soil enzymes was studied: catalase, urease, dehydrogenase, protease and cellulase.

The activity of the enzyme catalase was determined by the gasometric method by Galstyan (1974). The essence of the method is to determine the amount of oxygen released during the decomposition of hydrogen peroxide. The activity of the enzyme invertase was determined by a modified colorimetric method of Khaziev (1957). The essence of the method is to determine the optical density of the solution after the reduction of copper by glucose and fructose, released during the hydrolysis of sucrose. Urease activity was determined by the colorimetric method of Scherbakova (1983), by determining the amount of ammonium released using Nessler's reagent. Dehydrogenase activity was determined by Galstyan (1962), by photocolometric determination of the amount of formed

triphenylformazan (TFF). Protease activity was determined by the method of Galstyan-Harutyunyan (1976), a method based on the ability of proteases to decompose the protein substrate into amino acids, followed by photolorimetric determination of their amount using ninhydrin. Cellulase activity was determined based on the ability of the enzyme to decompose biopolymers to glucose, the amount of which is determined iodometrically by back titration with sodium hyposulfite (Khaziev, 2005; Titova, 2012).

Mathematical analysis of the data were performed with Microsoft Excel 2010 and Statgraphics 18.1 trial.

Table 4. Technological operations for the period 2018-2020, variant intensive farming system

2018	2019	2020
- collection of the predecessor in 2017	- application of Ammonium Sulfate 100 kg/ha	- harrowing
- disking (12-15 cm)	- cultivation (12-15 cm)	- sowing of corn together with introduction of a diamophos of 125 kg/hectare 9:25:25
- deep loosening 35-37 cm (autumn)	- harrowing	- introduction of soil herbicide
- application of urea 250 kg / ha (spring)	- sowing of sunflower with the introduction of complex fertilizers 115 kg/ha 8:24:24	- care 1: application of insurance herbicide + foliar fertilization (3-5 leaves)
- cultivation (12-15 cm)	- introduction of soil herbicide	- care 2: foliar fertilization (7-8 leaves)
- discusing (8-10 cm)	- care 1: herbicide around the perimeter of the field and inter-row tillage	- care 3: application of insecticide (on the panicle)
- sowing of corn together with introduction of a diamophos of 120 kg/hectare 10:26:26	- care 2: application of graminicide, fungicide, growth regulator and feeding on the leaves (4-5 pairs of true leaves)	- harvesting by direct combining
- introduction of soil herbicide	- care 3: application of insecticide, fungicide and foliar fertilization (asterisk)	- disking (12-15 cm)
- care 1-2: application of insurance herbicide + foliar fertilization	- harvesting by direct combining	- deep loosening (35-37cm)
- care 3: application of insecticide (on the panicle)	- disking (12-15 cm)	- application of urea 250 kg/ha
- harvesting by direct combining	- deep loosening (35-37cm)	- cultivation (12-15 cm)
- disking (12-15 cm)	- application of urea 250 kg/ha	
- plowing (25-28 cm)	- cultivation (12-15 cm)	

## RESULTS AND DISCUSSIONS

Analyzing the obtained data (Table 5) should pay attention to a significant decrease in invertase activity in the conditions of intensive farming system compared to the rest of the studied options, especially in layers 0-10 and 10-20 cm. The most significant difference was recorded in 0-10 cm layer. The highest values of invertase activity are characterized by

variants of fallow and organic farming system with the use of green manure. Similar data were reported by the researcher Mao-hua, 2012. We compare the obtained activity data of all enzymes with the comparative scale (Table 7) proposed by Zvyagintsev (1978). According to Table 7, all studied soils have a medium degree of invertase activity.

Soils involved in organic farming have the highest rates of urease enzymatic activity, which is confirmed by Meysner, 2013. The reason for this is the periodic application of organic fertilizers and the use of green manures. The lowest activity of this enzyme was in the variant of intensive farming system. High urease activity in the variant of intensive technology is a consequence of the introduction of high doses of urea in the cultivation of corn. This leads to an increase in urease activity at a depth of 10-20 cm.

The studied soils are characterized by an average degree of catalase activity. However, it is worth noting the significant difference between all the options studied, in particular, the highest rates were recorded in the variant of the organic farming system with the use of green manure, and the lowest in the variant of the intensive farming system. Different agricultural uses of soils lead to significant changes in the activity of catalase and many other enzymes (Kuscu, 2018; Petcu, 2014).

The highest protease activity is characterized by the variant of fallow, and the lowest - the variant of intensive farming system where the activity of this enzyme in the 0-10 cm layer is more than 4 times lower. A large percentage of cereals in crop rotation causes a decrease in protease activity. In general, it should be noted that the most significant difference between the studied variants is observed in the layers of 0-10 and 10-20 cm. Among the variants of the organic farming system, the variant with the use of vetch yara is characterized by higher prosthesis activity. The degree of protease activity is average in the variant of intensive farming system and very high in other variants. The thesis of increasing the activity of soil enzymes, especially proteases, under the conditions of the organic system of agriculture, has been reported in numerous other studies by other authors (Kwiatkowski, 2020; Melero, 2004; García-Orenes, 2016).

All variants have a high degree of dehydrogenase activity in the 0-10 cm layer. The exception is the variant of the intensive system of agriculture, which has a medium level. There was an increase in dehydrogenase activity in the layers of 20-30 and 30-40 cm and vice versa decrease in 0-10 and 10-20 cm in the variant of intensive farming system. Intensive farming system causes a decrease in dehydrogenase activity, and in organic farming, on the contrary - an increase, which also is confirmed by the data Kobierski, 2020 also. There is information in the literature that cellulase activity is a fairly stable indicator that most likely responds to the amount of substrate entering the soil (Doyle, 2006; Cenciani, 2011). Analyzing the obtained data, we note the weak cellulose-destroying activity of all studied soils. The highest values are in fallow, and the lowest – in the organic system using of compost. Also, an interesting fact is the increase in cellulase activity in the layer of 10-20 cm within the variant of organic farming system (green manure) and a drop in the variant of intensive

farming system. In our opinion, the small difference between the indicators of cellulase activity in the studied soils also indicates a greater influence of the quantity and quality of organic matter entering the soil than the system of agriculture.

The analysis of the obtained data (Table 5) shows that the soil samples from the organic system are characterized by a higher biological activity for almost all these indicators and in all layers. On the contrary, the soil from the variant of the intensive growth system is characterized by a decrease in biological activity in the layer of 0-10 cm and an increase in the indicators in the layer of 30-40 cm. According to Two-way ANOVA analysis of farming system and soil depth on soil enzyme activity (Table 6), both factors have a significant effect on the activity of soil enzymes, with the exception of cellulase. However, the value of F indicates that the effect of depths is much higher (except for catalase).

Table 5. Enzymatic activity of chernozems under different farming system, the average for the years 2018-2020

Variant	Depth, cm	Catalase, cm <sup>3</sup> O <sub>2</sub> per 1 g of soil for 1 min	Dehydrogenase, mg of TFF per 10 g of soil for 24 hours	Invertase, mg of glucose per 1 g of soil for 24 hours	Urease, mg NH <sub>3</sub> per 10 g of soil for 24 hours	Protease, mg of glycine per 1 g of soil for 24 hours	Cellulase, µg of glucose per 10 g of soil for 48 hours
green manure	0-10	7.54a	11.68a	29.89a	25.61a	17.10a	5.93a
		0.25	0.43	0.70	1.51	1.40	0.44
	10-20	7.36	9.42ab	20.94b	18.54b	5.41	5.84a
		0.28	0.42	0.35d	1.63	0.36	0.30
	20-30	7.00	6.11c	12.82c	14.03c	3.59	2.60b
		0.23	0.22	0.97	1.29	0.32	0.21
	30-40	5.79ab	4.27d	7.78d	12.20d	4.08a	2.09b
		0.27	0.20	0.48	1.23	0.21a	0.18
compost	0-10	6.35a	11.83a	22.47a	16.07a	11.65	6.2
		0.20	0.49	0.70	1.28	0.72a	0.49a
	10-20	6.24ab	9.37b	18.83b	16.15b	5.91	5.22
		0.24	0.46	1.06	1.46	0.39	0.36
	20-30	6.12ab	7.32c	11.45c	13.28c	3.55b	2.60b
		0.22	0.43	0.41	1.08	0.24	0.18
	30-40	5.46ab	5.89d	8.44d	11.93d	2.44c	1.76c
		0.21	0.35	0.41	0.14	0.17	0.14
Fallow	0-10	5.63a	12.38a	35.83a	14.61a	21.96a	6.38a
		0.30	0.47	2.35	1.23	1.29	0.44
	10-20	4.92ab	9.92b	19.41b	11.96b	9.70b	5.30b
		0.20	0.35	1.54	1.09	0.53	0.46
	20-30	5.01	7.82c	15.56	12.05c	3.81	3.08
		0.21	0.27	1.26	1.21	0.33	0.25
	30-40	4.39ac	4.72d	9.40c	10.47d	2.04c	1.86c
		0.17	0.15	0.71	1.12	0.16	0.12
Intensive	0-10	4.28a	8.88a	15.92a	13.71	4.71	6.13a
		0.23	0.41	0.94	0.94	0.51	0.45
	10-20	4.03	9.11ab	16.12	14.60	3.65a	3.76
		0.21	0.51	0.84	1.30	0.26	0.44
	20-30	3.97	8.68	15.78	12.34	3.23	3.31
		0.17	0.36	1.02	1.16	0.24	0.22
	30-40	3.56ac	6.85a	10.65	12.20a	1.70b	2.34c
		0.17	0.38	0.65	1.27	0.14	0.15

Notes. The numerator is the average value; denominator – standard error. The letters in the columns of variants are significant differences between different layers of soil (p < 0.05).

Table 6. A two-way ANOVA for the effects of farming system and soil layers on soil enzyme activities

Influence factor		Catalase	Dehydrogenase	Invertase	Urease	Protease	Cellulase
Farm system	F	130.62	21.47	13.61	3.76	62.41	0.81
	P	0.0000	0.0000	0.0000	0.0000	0.0000	0.4912
Soil Layer	F	18.79	181.62	16.78	168.38	310.59	135.20
	P	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Interaction	F	1.20	17.46	3.75	10.29	35.57	2.77
	P	0.2925	0.0000	0.00001	0.0000	0.0000	0.0037

Table 7. Scale of comparative assessment of soil enzymatic activity (Zvyagintsev, 1978; Titova, 2012)

Enzyme activity	Catalase, cm <sup>3</sup> O <sub>2</sub> per 1 g of soil for 1 min	Dehydrogenase, mg of TFF per 10 g of soil for 24 hours	Invertase, mg of glucose per 1 g of soil for 24 hours	Urease, mg NH <sub>3</sub> per 10 g of soil for 24 hours	Protease, mg of glycine per 1 g of soil for 24 hours	Cellulase, µg of glucose per 10 g of soil for 48 hours
Very low	<1	<1	<5	<3	<1	<10
Low	1-3	1-3	5-15	3-10	1-3	10-20
Medium	3-10	3-10	15-50	10-30	3-5	20-50
High	10-30	10-30	50-150	30-100	5-8	50-100
Very high	>30	>30	>150	>100	>8	>100

## CONCLUSIONS

Agricultural using of soils leads to significant changes in their biological activity. The nature and extent of these changes depend on the system of agriculture, crop rotation and fertilization system. This once again confirms the significant sensitivity of soil enzymatic activity to any anthropogenic impact.

The data obtained within our studies indicate a decrease in the activity of such enzymes: invertase, protease, dehydrogenase and cellulase under conditions of agrogenic use of typical chernozems. But, at the same time, the activity of urease and catalase increases. The enzyme activity changes with soil depth in accordance with the content of organic matter and microbiological activity. So, the activity all studied enzymes usually decreases with soil depth. In the variant of intensive farming system, as a result of plowing, homogenization of a 0-30 centimeter layer of soil is observed, which leads to equalization of indicators at these depths. A feature of the variant of the intensive farming system is an increase in the activity of urease at a depth of 10-20 cm, which is a consequence of the introduction of urea. The application of organic fertilizers in the variants of the organic system of agriculture (especially the use of green manure) increases the activity of all studied enzymes in comparison with the variant of the intensive system of agriculture.

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## RESEARCH OF VECTOR MONITORING OF SOIL PROFILES IN IALOVENI DISTRICT BASED ON DIGITALIZED MATERIALS

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### Abstract

*In the study, the archaeological pedological materials were selected on 25 communes from Ialoveni rayon at 1:10000 scale. The materials were scanned and digitally connected to the national reference system MoldRef-99. The contours of the soil were then vectorized and the attributive information was provided for each contour. These works were performed at a scale of 1:10000. As a result, 4050 soil contours were erected on an area of 57577.10 ha (agricultural land). It is very clear that the predominance of the chernozems' soil cover structure with 62.88% of the agricultural soils, the higher share of the carbonate, levgated and ordinary subtypes. The surface of carbonate soils has virtually doubled (from 9666 ha - first cycle to 17162 ha - cycle II). The second place occupies the gray soils by 13.96%. Alluvial soils are the next step with 8.98%. About 14% of the remaining soils, including 5.1% under landslides. In the works, the general digital layer of soil profiles and soil profiles according to genetic horizons has been created which includes the specific attributive parameters. In the Ialoveni district the morphological and analytical data for 540 profiles and 2831 genetic horizons were introduced.*

**Key words:** agricultural land, digital map of the soils cover, Ialoveni district, soil profile, vectoring.

### INTRODUCTION

Soil represents the main natural wealth of the Republic of Moldova.

After the composition and natural fertility, the soils of Moldova are part of the most valuable resource category, characterized by a remarkable diversity, related to the variations of the local horizontal and vertical zones, climatic and geological conditions.

One of the main objectives of the Republic of Moldova is the long-term preservation of the quality of the soil cover, together with the protection of the environment.

The level of soil quality depends to a large extent on the productivity of agricultural crops, the development of the livestock sector, the export of agri-food products, the welfare of the population and the ecological situation in the country. Intensive exploitation of soil resources worldwide has caused degradation in recent decades.

Taking into account the global trends, the degradation rates and the stranded losses of agricultural areas, as well as the development of agriculture, the problem of maintaining the quality of the soil cover on agricultural land becomes for our state a strategic concern of

national security. Soil degradation in the Republic of Moldova is one of the most serious problems present at the present stage.

The main forms of degradation of the soil cover are: water erosion, dehumification, soil clearing in nutrients, excessive destructuring and compacting, solonetization, salting, and so on. Degradation processes and non-compliance with agricultural technologies have caused declining production capacity of soils. For imaging the soil (as a resource) in two-dimensional format, the system uses a series of real-world objects through which its description is performed.

Such objects refer to: - soil profile; - horizons and profile layers; - soil areas; - experimental (pedological) sectors.

The use of these data widens the use of geospatial information for the management of other branch and regional core information resources and their components, ensuring integration with other thematic geospatial data. The system uses the geospatial database model, in which the data presents real spatial object models.

Graphical data is retained as attributes corresponding to spatial objects structured in

appropriate classes and are held in three-dimensional format:

- vector data for the presentation of spatial objects;
- raster data for the presentation of continuous seamless images, network thematic data and surfaces;
- addresses and locators to find the geographic situation.

When viewing objects according to the image generation rules for each of the discrete stairs, appropriate conditions are provided for reflecting the object.

Each scale of object reflection is a combination of geometric primitives: conventional symbols, explanatory inscriptions, and a series of graphical signs: color, size, thickness, discontinuity, hatching, background, font, and so on.

In the real world, the notion of "soil profile" is defined as a slice section of the soil, from its surface to the parental rock, which demonstrates the vertical consecutivity of the sequentially related and logically sequential genetic layers (soil horizons). The profile is described through soil horizons, characterizing them according to morphological criteria (internal), composition and properties.

It can also be described by layers - the depth and sampling interval, which do not depend on ground horizons.

By examining the soil profile as a whole, it is described by a series of inherent properties: the soil type (according to soil classification), the soil profile structure, the depth of the profile, the presence and depth of the groundwater, the depth of penetration roots etc. (Baumgardner, 1988).

Viewing geographic information or geographic view of information allows people to notice changes and even forecasting reality. Applications on virtual reality use representations of real spatial phenomena, but also of non-formal phenomena, simply because the human brain is accustomed to solving problems in three-dimensional space. Important parts of the software component and the data required for configuring and populating cyberspace will be taken from geoprocessing applications, digital geodata archives, and current data flows. Similarly, space research will benefit both from "real space" and from cyberspace applications.

## MATERIALS AND METHODS

In the study, the archaeological pedological materials were selected on 25 communes from Ialoveni district at 1:10000 scales. The materials were scanned and digitally connected to the national reference system MoldRef-99. The contours of the soil were then vectorized and the attributive information was provided for each contour. These works were performed at a scale of 1: 10000.

## RESULTS AND DISCUSSIONS

As a result, 4050 soil contours (Figure 1) were erected on an area of 57577.10 ha (agricultural land). It is very clear that the predominance of the chernozems' soil cover structure with 62.88% of the agricultural soils, the higher share of the carbonate, levigated and ordinary subtypes. The surface of carbonate soils has virtually doubled (from 9666 ha - first cycle to 17162 ha - cycle II). The second place occupies the gray soils by 13.96%. Alluvial soils are the next step with 8.98%. About 14% of the remaining soils, including 5.1% under landslides.

For the updating of the pedological map were used: the Ialoveni district soil digital map (the first pedological mapping cycle) elaborated on the basis of IPAPS materials "Nicolae Dimo" at scale 1:50000, the digital map of the soils cover on the communes Ialoveni district (second cycle of pedological mapping) developed on the basis of IPOT materials at scale 1:10000 and analytical data of soil profiles. After mapping the mapping materials, updating and correcting them, the common digital map of the soils was developed. Based on this map of the soil surface, the land under the water category, the mound subcategory, the not agricultural not agricultural land category and the full use of infrastructure based on the digital map of the land infrastructure in 2016 were excluded. The structure of the soil cover was divided for each sub-category/land use category.

In the works, the general digital layer of soil profiles and soil profiles according to genetic horizons has been created which includes the specific attributive parameters.

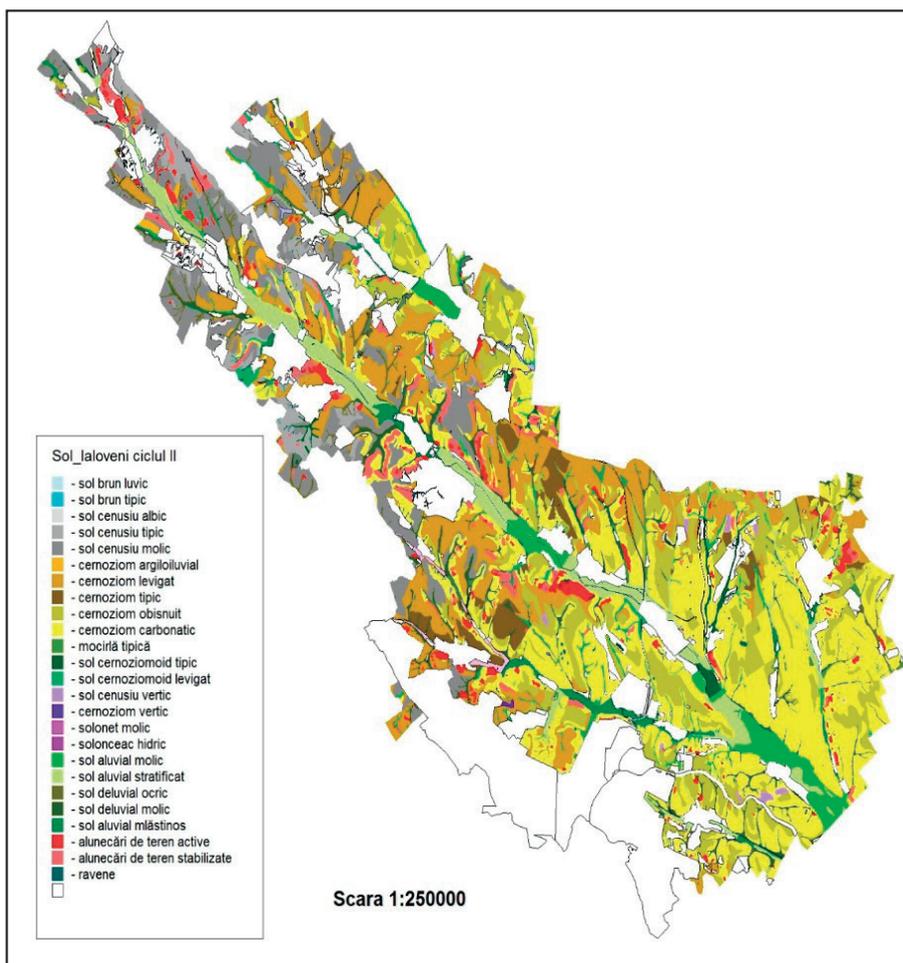


Figure 1. The digital soil maps (II mapping cycle)

On the second place are the gray soils (18.54%) represented by the soft ones with 10.82% and typical with 7.15% of the soils. The area of alluvial soils is 9.01%.

Landslides are 6.65%. The rest of the soils cover an area of about 9%. The weighted average credit rating on the rayon is 66.31 points.

In the Ialoveni district the morphological and analytical data for 540 profiles (Figure 2) and 2831 genetic horizons were introduced.

As a result of the work carried out within the object, 59820 contours (Figure 3) have an area of 66017.51 ha, compared to 2418 of the original contours covering an area of 72632.29 ha.

In the structure of the soil cover the chernozems predominate with about 56%, where the leachates prevail with 19.33%, the usual (16.88%), the carbonate with 13.30%.

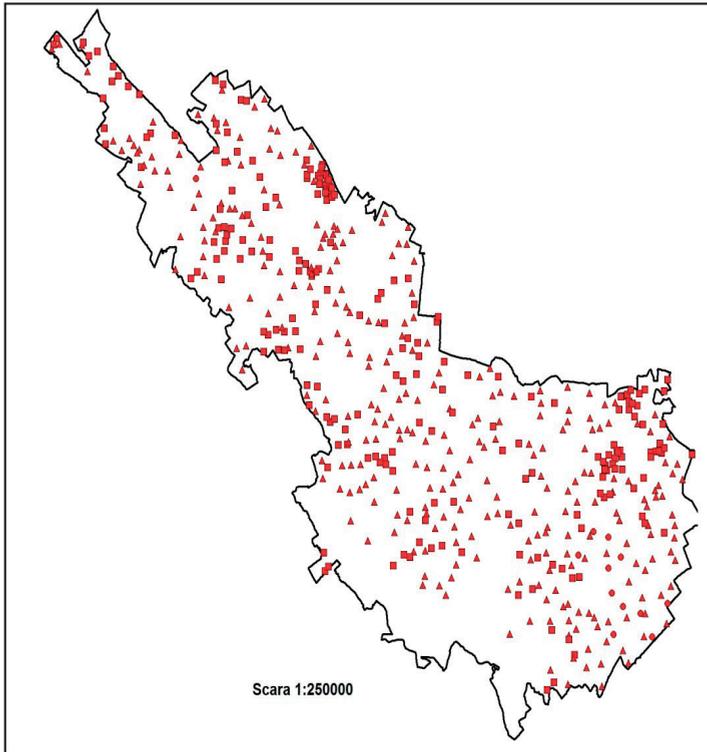


Figure 2. Location of soil profiles

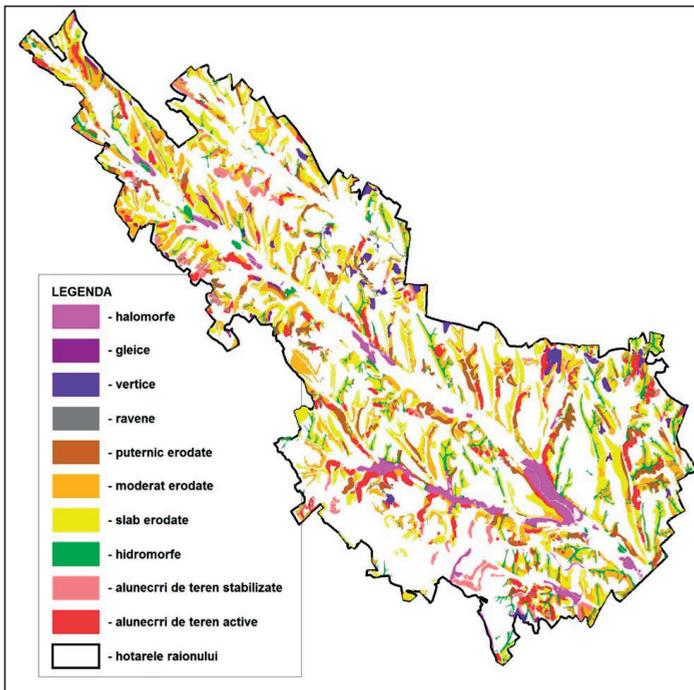


Figure 3. Degraded Soil Map

## CONCLUSIONS

Chernozem subtypes predominant in the composition of the ravine soil cover are the most extensively subjected to degradation by water erosion. The surface of these soils amounts to 16536.51 ha, which represents 82.45% of the eroded land. The most affected are carbonate chernozems with 69.04%. More than 16% of eroded soils hang on gray soils.

The landslides have become widely spread. The area of lands lands or lands up to 4387.65 ha or 6.65% of the territory due to the intensive land use of landowners, which makes it impossible to carry out complex protection works.

The analysis shows that about 25089.96 ha (38%) of the total of 66017.51 ha of the soils of the Ialoveni district are subjected to various degradation processes.

The soil and laboratory pedological works have determined: humus content, texture, degree of solonization, carbonate content and depth, degree of erosion, current reaction, hydrolytic acidity, parental rock and so on.

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## THE EFFECT OF APPLYING ON AGRICULTURAL LAND OF THE COMPOST FROM SEWAGE SLUDGE ON THE SOIL AND THE MAIZE CROP

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### Abstract

*The compost obtained from sludge from wastewater treatment being an important source of macro and micronutrients, can be used in agriculture, because it reduces the production costs and improves the soil quality by providing nutrients and organic matter necessary for modern, ecological agriculture, in the conditions to improve the capacity to retain moisture in the soil, reducing the pressure on the environment generated by the storage of this waste. The compost used in the experiments is suitable for the use in agriculture without risks of environmental and soil pollution, in compliance with the rules in force. The obtained results show that by applying the compost produced at SEAU Mioveni, even in the variants where the highest doses (60 t/ha) were applied, there are no significant changes in the chemical properties of the soil, especially the content of heavy metals. The values determined in the soil after applying the compost to all the experienced variants are far below the maximum allowed values for the concentrations of heavy metals in the soils. Also, analyzing the results regarding the risk of translocation of different chemical elements in the maize grains, it can be seen that, in general, all indicators register values well below the limits from which zootoxicity phenomena can occur. No increases in heavy metal contents in the maize grains are observed as the doses of compost used increase.*

**Key words:** compost, sludge, soil, maize.

### INTRODUCTION

The changes in the soil as a result of applying these residues are registered by the agrochemical state, the agrophysical state, the agrobiological state, all competing in defining the soil fertility.

The positive effect of the organic matters in general, and those from urban activity, in particular, on the physical, chemical and biological properties of the soil is also reflected in plant production, which in most cases is increasing.

The organic matter is directly involved in the retention of heavy metals, being one of the first metals studied in this regard (Kiikkila, 2002) showing that biosolid is an immobilizing agent of this heavy metal.

On the other hand (Moolenaar and Beltrami, 1998) proved that heavy metals can also be complexed by the dissolved organic matter, which influences the ion balance. One of the main factors involved in the absorption of heavy metals is the soil pH, their accessibility being very low in the reaction range of 6.5-7.

The presence of competitive metal ions can affect the adsorption of heavy metals in soils. Ca<sup>2+</sup> ions interfere in the adsorption processes with Zn, Cd, Cu, as a result of the fact that Zn and Cd ions are retained in the soil by cationic exchange reactions, while Cu and Pb form organic complexes with oxides of Fe, Al and Mn (Kiekens, 1983; Pirangeli et al., 2001, 2003).

The adsorption of heavy metals by iron oxides is accompanied by a protonation being dependent on pH, according to research conducted by Maizeell & Schwetmann (1996)

The positive effects are due both to the high content of organic matter and nutrients in forms accessible to plants, and to the improvement of the processes of structuring the elementary soil particles in hydrostable aggregates, to the increase of water retention capacity.

The concentration of heavy metals is among the most important factors restricting the use of urban waste products on agricultural land, due to their potentially negative effects on plant biomass and their translocation into food.

The data in the literature contain different ways of interpreting the contents of heavy metals in soils, specifying limit values, but it seems that the closest model of reality is the one that takes into account the content of total forms in the soil (EPA, 1993).

The current acidity of the soil registered a tendency of reduction by biosolid fertilization in the years of application and remanence.

The potential acidity followed the same variation as the current one, so that in the conditions of applying biosolid and in the first year of remanence it had a tendency to decrease as later there was an update in the second year of remanence (Trașcă, 2008).

The soil in the location of the experiment is part of the group of luvosol, podzolic, pseudogley type, as a result of their formation under the vegetation of the quercineae forest, under the conditions of a dominant lithology of fine-textured clays and located on relatively flat-horizontal terrain (Trașca, 2008).

The increased interest in fertilizing the soil with sludge resulting from urban wastewater has been manifested since 1970, when it was established that it can be considered an organic fertilizer (Tomlin, 1993). The use of sludge resulting from urban wastewater treatment in agriculture is dependent on the properties of the soil, of which pH, organic matter and nutrient content occupy a preferential place, but being restricted by the presence of heavy metals especially Cd, Pb, and Ni, whose concentration in the environment is governed by the nature of the element and the dose applied (Lopez-Mosquera, 2000).

The effect of sludge from urban wastewater treatment on the soil is investigated both in terms of pedo-improvement and in terms of environmental impact. As Beltran (1999) pointed out, knowledge of the chemical composition of sludge is of particular importance when making recommendations on application rates on the agricultural land.

Over time, soluble organic compounds tend to turn into insoluble forms, with the amount of heavy metals settling to low values when the bioavailability decreases (McBride, 1995).

Researches on the effect of sludge application on the soil have not exceeded 30 years, as demonstrated by numerous works (Kabata-Pendias, 2004).

## MATERIALS AND METHODS

In order to study the influence of the application of compost resulting from the sludge proceeding from the treatment plant on the agricultural crops and on the soil, maize was used as a test plant, the sown hybrid was F376.

The basic work of the soil was ploughing, carried out at 25 cm, the preparation of the germination bed was done by two passes with a disc harrow, the sowing was done with SPC 6, and the seed rate used was 20 kg/ha.

The experience included 5 experimental variants in 3 repetitions, the surface of an experimental plot was 105 m<sup>2</sup>.

The experimental variants were:

V<sub>1</sub> - Control;

V<sub>2</sub> - 10 t/ha;

V<sub>3</sub> - 20 t/ha;

V<sub>4</sub> - 40 t/ha;

V<sub>5</sub> - 60 t/ha.

The administration of the compost in the specific doses of each variant was performed by manual spreading and incorporated in the soil by ploughing.

Weed control was done by herbicidation using Dual Gold 960EC herbicide, at a dose of 1.6 l/ha, applied pre-emergently.

### **The quality of the compost used in the experiments**

In order to determine whether the compost produced at SEAU Mioveni can be used in the experiments, its main chemical characteristics were determined (Table 1).

The qualitative parameters of the analyzed compost are within the maximum allowable values provided for use in agriculture, including in terms of heavy metal content.

The samples of compost, soil and plant (leaves, grains) were taken and analyzed according to the methodology in force (pH was determined potentiometrically in aqueous suspension; the organic matter was determined by Walkley-Black-Gogoasă method; mobile phosphorus and potassium by Egner-Riehm-Domingo method; total nitrogen by Kjeldahl method; heavy metal content, in total forms, with dosing by atomic absorption spectrophotometry).

Table 1. The main chemical characteristics of compost

No.	The quality indicator	U.M.	Value	Maximum values (Ord. 344/2004)
1	Volatile substances	%	35.34	-
2	pH	-	7.09	-
3	C <sub>organic</sub>	% s.u.	21.5	-
4	N <sub>total</sub>	% s.u.	1.52	-
5	P <sub>2</sub> O <sub>5</sub>	% s.u.	1.38	-
6	K <sub>2</sub> O	% s.u.	0.675	-
7	CaO	% s.u.	0.35	-
8	Cadmium	mg/kg s.u.	1.04	10
9	Chromium	mg/kg s.u.	44.8	500
10	Copper	mg/kg s.u.	74.3	500
11	Nickel	mg/kg s.u.	26.5	100
12	Lead	mg/kg s.u.	46.3	300
13	Zinc	mg/kg s.u.	612	2000
14	Cobalt	mg/kg s.u.	6.34	50
15	Arsenic	mg/kg s.u.	4.09	10
16	Total coliform bacteria	probable no./g s.u.	1352400	-
17	Fecal coliforms	probable no./g s.u.	236523	-
18	Enterococci	UFC/g s.u.	105840	-

## RESULTS AND DISCUSSIONS

### The influence of compost fertilization on the soil

The obtained results show us that by applying the compost produced at SEAU Mioveni, on the agricultural land, even in large quantities (60 t/ha), there are no significant changes in its

chemical properties, and especially in the case of potentially polluting heavy metals.

The values determined in soil after the application of compost, in all experimental variants are well below the maximum allowed values for the concentrations of heavy metals in soils (Table 2).

Table 2. The values determined in the soil after application of the compost

No.	The analyzed parameter	V <sub>1</sub>	V <sub>2</sub>	V <sub>3</sub>	V <sub>4</sub>	V <sub>5</sub>
1	pH	5.73	5.96	5.82	5.92	6.02
2	Organic matter content (%)	4.59	4.28	4.43	4.13	4.28
3	Soluble salts (%)	0.022	0.019	0.023	0.019	0.017
4	Water storage capacity (%)	53	52	53	53	52
5	Apparent density (g/cm <sup>3</sup> )	1.24	1.28	1.24	1.25	1.28
6	C total (% s.u.)	1.07	1.22	1.13	1.15	1.11
7	N <sub>total</sub> (% s.u.)	0.126	0.122	0.128	0.125	0.128
8	P <sub>2</sub> O <sub>5</sub> (% s.u.)	0.110	0.111	0.105	0.114	0.115
9	K <sub>2</sub> O (% s.u.)	0.199	0.277	0.239	0.207	0.211
10	CaO (% s.u.)	0.153	0.155	0.157	0.143	0.128
11	Cadmium (mg/kg s.u.)	0.602	0.546	0.395	0.376	0.508
12	Chromium (mg/kg s.u.)	34.28	36.88	36.38	32.03	31.28
13	Copper (mg/kg s.u.)	17.32	16.29	16.42	15.44	15.70
14	Nickel (mg/kg s.u.)	26.23	25.03	24.79	23.16	22.46
15	Lead (mg/kg s.u.)	13.68	12.03	13.50	11.60	14.40
16	Zinc (mg/kg s.u.)	59.99	59.92	55.58	53.79	54.31
17	Cobalt (mg/kg s.u.)	11.27	10.77	10.87	10.50	10.62
18	Arsenic (mg/kg s.u.)	0.058	0.036	0.032	0.049	0.055
19	Total coliform bacteria (probable no./g s.u.)	173450	2855	23404	7357	5946
20	Fecal coliforms (probable no./g s.u.)	0	0	0	0	0
21	Enterococci (UFC/g s.u.)	0	72	0	300	44

## Soil chemical analysis

It is found that by applying the compost produced at SEAU Mioveni, on the agricultural land, even in the fertilized variants with the highest doses (60 t/ha), there are no significant changes in its chemical properties and with

special reference to the potentially polluting heavy metals. The values determined in the soil after the maize harvest, in all the experimented variants, are far below the maximum allowed values for the concentrations of heavy metals in the soils (Table 3).

Table 3. Soil chemical characteristics after maize harvesting

No.	The analyzed parameter	V <sub>1</sub>	V <sub>2</sub>	V <sub>3</sub>	V <sub>4</sub>	V <sub>5</sub>
1	pH	6.16	6.10	6.04	6.07	6.16
2	Organic matter content (%)	4.36	3.77	3.76	3.96	4.06
3	Soluble salts (%)	0.021	0.018	0.015	0.022	0.019
4	Water storage capacity (%)	40.7	41.1	40.5	40.9	41.3
5	Apparent density (g/cm <sup>3</sup> )	1.28	1.28	1.30	1.29	1.28
6	C total (% s.u.)	1.27	1.08	1.17	1.18	1.20
7	N <sub>total</sub> (% s.u.)	0.105	0.097	0.098	0.097	0.102
8	P <sub>2</sub> O <sub>5</sub> (% s.u.)	0.126	0.123	0.119	0.112	0.121
9	K <sub>2</sub> O (% s.u.)	0.59	0.58	0.58	0.57	0.60
10	CaO (% s.u.)	0.26	0.24	0.25	0.26	0.26
11	Cadmium (mg/kg s.u.)	0.766	0.747	0.554	0.364	0.589
12	Chromium (mg/kg s.u.)	55.96	53.18	54.39	53.32	50.65
13	Copper (mg/kg s.u.)	18.58	17.87	17.62	16.24	17.13
14	Nickel (mg/kg s.u.)	26.26	22.49	24.80	23.21	21.66
15	Lead (mg/kg s.u.)	17.90	16.67	19.42	16.83	18.27
16	Zinc (mg/kg s.u.)	67.36	60.99	66.68	65.42	62.48
17	Cobalt (mg/kg s.u.)	12.78	11.83	12.03	11.99	11.65
18	Arsenic (mg/kg s.u.)	0.046	0.039	0.035	0.041	0.051
19	Total coliform bacteria (probable no./g s.u.)	17876	38.454	178438	1.025	101108
20	Fecal coliforms (probable no./g s.u.)	39	100	7.763	241	5957
21	Enterococci (UFC/g s.u.)	0	0	278	0	0

In Romania, the technical norms regarding the protection of the environment, and especially of the soils, when sewage sludge are used in agriculture (even composted) were provided in the Order 344/2004, published in the Official Gazette no. 959/October 19<sup>th</sup>, 2004.

The technical norms of the Order 344/2004 have as main provision the content of heavy metals, both from the soils on which the sewage sludge is applied, and the content of these metals in the sludge.

These norms aim at capitalizing on the agrochemical potential of the sludge from the treatment plants, preventing all harmful effects

on the soils, considered the basic link in the soil-plant-animal (human) trophic chain. The references are for the following heavy metals: *cadmium copper, nickel, lead, zinc, mercury and chromium*, focused on 3 directions:

- the maximum permissible values of heavy metals in the soils on which the sewage sludge is applied (Table 4);
- the maximum allowed values of heavy metals from the sewage sludge to be applied on the soils (Table 5);
- the limit values for the annual quantities of heavy metals accumulated in the soils (Table 6).

Table 4. The maximum permissible values for the concentrations of heavy metals in soils

The analyzed parameter	The limit value (mg/kg s.u.)
Cadmium	3
Copper	100
Nickel	50
Lead	50
Zinc	300
Mercury	1
Chromium	100

Table 5. The maximum permissible concentrations of heavy metals in the sewage sludge for use in agriculture

The analyzed parameter	The limit value (mg/kg s.u.)
Cadmium	10
Copper	500
Nickel	100
Lead	300
Zinc	2000
Mercury	5
Chromium	500
Cobalt	50
Arsenic	10

Table 6. The limit values for the annual quantities of heavy metals that can be introduced into agricultural land based on a 10-year average for use in agriculture

The analyzed parameter	The limit value (kg/ha/an)
Cadmium	0.15
Copper	12
Nickel	3
Lead	15
Zinc	30
Mercury	0.1
Chromium	12

### The influence of compost fertilization on maize crop

The analyzes performed on the maize leaves show that all the analyzed indicators do not register values in general which are phytotoxic for the maize plants fertilized with compost from sewage sludge.

There are there slight increases only in copper and zinc, at high doses of compost, compared

to the unfertilized witness, but without affecting the normal growth, development and fruiting of maize plants (Table 7).

It is necessary to follow the way in which the translocation of different chemical elements in the maize grains took place, by analyzing their content, after harvesting and interpreting these values in correlation with the contents determined in the leaves.

Table 7. The influence of compost fertilization on maize cultivation

No.	The analyzed parameter	V <sub>1</sub>	V <sub>2</sub>	V <sub>3</sub>	V <sub>4</sub>	V <sub>5</sub>
1	Humidity (%)	78.4	76.1	75.63	76.1	78.2
2	Cadmium (mg/kg s.u.)	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3
3	Chromium (mg/kg s.u.)	< 1.5	< 1.5	< 1.5	< 1.5	< 1.5
4	Copper (mg/kg s.u.)	6.44	4.70	11.71	9.02	8.40
5	Nickel (mg/kg s.u.)	< 1.5	< 1.5	< 1.5	< 1.5	< 1.5
6	Lead (mg/kg s.u.)	< 1.5	< 1.5	< 1.5	< 1.5	< 1.5
7	Zinc (mg/kg s.u.)	17.22	13.60	50.54	48.57	44.0
8	Cobalt (mg/kg s.u.)	< 1.5	< 1.5	< 1.5	< 1.5	< 1.5
9	Arsenic (mg/kg s.u.)	0.043	0.030	0.030	0.037	0.039
10	Total coliform bacteria (probable no./g s.u.)	32	2381	880	1627	2607
11	Fecal coliforms (probable no./g s.u.)	0	23	18	8	8
12	Enterococci (UFC/g s.u.)	0	3	26	3	2

### Analysis of maize grains

The normal lead content of cereals is considered to be between 0.2-0.5 ppm, and the maximum in fodder is 40 ppm (Order MSF/MAAP-358/248 of 2003).

The normal copper content in the maize grains is 1.8 ppm and a maximum of 10 ppm in fodder (Table 8).

The fodders become toxic to animals when their cadmium content is 40-100 ppm.

Regarding zinc, the maximum content of fodder in this microelement is considered to be at 200 ppm.

Nickel is sometimes present in fodder, the normal value is below 5 ppm and the maximum accepted being situated at 12 ppm.

It can be seen that, in general, all the analyzed indicators register values well below the limits from which zootoxicity phenomena can occur.

Also, there is no increase in heavy metal content in maize grains, as the doses of compost used to fertilize maize increase.

It follows that from the point of view of the fodder quality of maize grains, it is not affected even in the case of the use of high doses of compost in the fertilization of the crop by the quality of the experienced one.

Table 8. Analysis of maize grains

No.	The analyzed parameter	V <sub>1</sub>	V <sub>2</sub>	V <sub>3</sub>	V <sub>4</sub>	V <sub>5</sub>
1	Humidity (%)	24.03	23.79	22.45	20.95	23.51
2	Cadmium (mg/kg s.u.)	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3
3	Chromium (mg/kg s.u.)	< 1.5	< 1.5	< 1.5	< 1.5	< 1.5
4	Copper (mg/kg s.u.)	1.19	1.19	1.18	1.25	1.09
5	Nickel (mg/kg s.u.)	< 1.5	< 1.5	< 1.5	< 1.5	< 1.5
6	Lead (mg/kg s.u.)	< 1.5	< 1.5	< 1.5	< 1.5	< 1.5
7	Zinc (mg/kg s.u.)	25.78	13.60	23.76	21.26	24.83
8	Cobalt (mg/kg s.u.)	< 1.5	< 1.5	< 1.5	< 1.5	< 1.5
9	Arsenic (mg/kg s.u.)	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03
10	Total coliform bacteria (probable no./g s.u.)	227	115	45	16	1052
11	Fecal coliforms (probable no./g s.u.)	0	0	0	0	0
12	Enterococci (UFC/g s.u.)	0	0	0	0	0

Also, in the maize crop, determinations were made regarding the influence of compost fertilization on: weeding degree, plant height, grain production, mass of one thousand grains, hectoliter mass (Table 9).

Determining the degree of weeding is important to see how it is influenced by the compost doses.

For each variant, three determinations were made, on each repetition, the presented data representing the average values.

It can be seen that the degree of weeding was influenced by the application of compost and the size of the doses used according to the experimental variants.

Thus, the degree of weeding increased by 19-70% in the variants fertilized with compost

compared to the witness variant (V<sub>1</sub>), which was not fertilized with compost. The highest increase in the degree of weeding, of 70%, is recorded in V<sub>4</sub> (40 t/ha compost).

This aspect is explained by the fact that in the composting process due to the high temperatures achieved in certain phases of composting, although most of the weed seeds are destroyed, there are still weed seeds with germination capacity.

As a result, a more careful management of humidity and air inside the compost pile is required so as to achieve the conditions for raising and maintaining temperatures corresponding to the destruction of pathogens and weed seeds.

Table 9. The influence of compost application on maize crop

The experimental variant	The degree of weeding (pl/m <sup>2</sup> )	Plant height (cm)	Production (kg/ha)	The mass of a thousand grains (g)	Hectoliter mass (kg)
V <sub>1</sub>	87	170	9125	348	73.8
V <sub>2</sub>	104	175	9675	352	70.4
V <sub>3</sub>	145	185	10147	348	71.9
V <sub>4</sub>	148	188	10285	336	72.3
V <sub>5</sub>	135	222	11814	370	88.0

The size of the plants had higher values by 3 to 30% for the variants fertilized with compost, compared to the unfertilized witness variant, the evolution of the height of maize plants registering the same increasing trend with the size of the compost doses used; thus the highest size of the maize plants (222 cm) was recorded in the fertilized version with the maximum dose of compost (V<sub>5</sub> = 60 t/ha).

The beneficial effect of compost on the vegetative growth of maize plants is very clear.

The maize grain production (average values, recalculated at STAS humidity, U = 14%)

There is generally a fairly high level of production due to climatic conditions in the experimental year, very favorable for maize crop.

The use of compost has determined very important increases in production, which reach 29% at V<sub>5</sub> (60 t/ha).

We mention the fact that the production increases obtained by using compost, although very important, are not very large, this due to the good natural fertility of the soil on which the experiments were located, as well as the fact that for reasons of economic efficiency and the witness without compost was fertilized with moderate doses of chemical fertilizers.

The mass of one thousand grains is in all variants within biological limits specific to the cultivated hybrid (F376), quite high, as a result of the achievement of favorable cultural and natural conditions for the witness variant as well.

There are no significant differences in the MMB of maize grains between the experimental variants.

The average values of the hectoliter mass registers relatively good values for the variants: V<sub>1</sub>, V<sub>2</sub>, V<sub>3</sub> and V<sub>4</sub> (MH = 70.4-73.8), but only at V<sub>5</sub> the MH value is higher (MH = 88). There are no significant differences in MH of maize grains between the experimental variants.

## CONCLUSIONS

The studied compost is suitable for use in agriculture without risks of environmental and soil pollution with strict compliance with the entire set of specific technical measures.

The application of compost produced on agricultural land, even at the maximum dose

(60 t/ha), did not cause significant changes on the chemical properties of the soil and with special reference to potentially polluting heavy metals.

The content of heavy metals determined in the soil when applying the compost and after harvesting the maize, in all the experienced variants is well below the maximum allowed values for the concentrations of heavy metals in the soils.

In general, all the analyzed indicators do not record values that are phytotoxic for maize plants fertilized with sewage sludge compost, regardless of doses.

There are slight increases only in copper and zinc, at high doses of compost, compared to the unfertilized witness, but without affecting the normal growth, development and fruiting of maize plants.

The results regarding the translocation of the different chemical elements in the maize grains highlight that in general, all the analyzed indicators register values well below the limits from which zootoxicity phenomena can occur.

Also, there is no increase in heavy metal content in maize grains, as the doses of compost used to fertilize maize increase.

From the point of view of the quality of maize grains, this is not affected even in the case of using high doses of compost to fertilize the crop by the quality of the experienced one.

The degree of weeding was influenced by the application of compost and the size of the doses used, as a result it requires a more careful management of moisture and air inside the compost pile so as to achieve the conditions of raising and maintaining temperatures for the total destruction of pathogens, but also weed seeds.

The analysis of the results on the influence of compost application on maize crop clearly highlights the beneficial effect of compost on maize plants.

The use of compost has determined very important increases in production, which reach 29% at V<sub>5</sub>, fertilized with 60 t/ha.

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## ABUNDANCE AND BIODIVERSITY OF INVERTEBRATES IN BROWN SOILS OF NATURAL AND AGRICULTURAL ECOSYSTEMS

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### Abstract

*The invertebrates' abundance and biodiversity of virgin and arable brown soils in the central zone of the Republic of Moldova have been investigated. Invertebrates sampling was carried out from test cuts by manual sampling of soil layers to the depth of soil fauna occurrence. The virgin brown soil in the natural ecosystem is characterized by a high abundance, biomass and biodiversity of the edaphic fauna compared to the soil under long-term agricultural use. A characteristic feature of the natural brown soils is the high concentration of invertebrates and Lumbricidae family in the upper layers of soils. Indicators of the edaphic fauna in soil profiles decreased with the depth. The relations between trophic levels are stronger in brown soils under forest, and trophic pyramids are more stable in the typical brown soil than in the luvic brown soil. The number and biomass of invertebrates in arable brown soils are lower by 2.8-3.4 times and by 2.2-7.5 times compared to brown soils of natural ecosystems. The number of edaphic fauna families decreased from 10-11 to 4-7. The disruption of trophic connections between invertebrates has been observed.*

**Key words:** biodiversity, soil invertebrates, typical and luvic brown soil, natural and agricultural ecosystem.

### INTRODUCTION

The primordial importance of the biodiversity for the environment maintaining stability and the stable development of communities is reflected in the Convention on Biological Diversity which was adopted by the United Nations Conference on Environment and Development in Rio de Janeiro on July 3-14, 1992 (Retrieved from <https://www.cbd.int/doc/legal/cbd-en.pdf>).

The Status of the World's Soil Resources (FAO, 2015) concluded that the loss of soil biodiversity is considered to be the one of the main global threats to soils in many regions of the world (Retrieved from <http://www.fao.org/documents/card/ru/c/c6814873-efc3-41db-b7d3-2081a10ede50/>).

Report FAO 2020 defines “soil biodiversity as the variety of life belowground from genes and species to the communities they form, as well as the ecological complexes to which they contribute and to which they belong, from soil micro-habitats to landscapes” (*State of Knowledge of Soil Biodiversity. Status, challenges and potentialities. Report 2020*. Retrieved from Report Soil Biodiversity.pdf).

Soil invertebrates' biodiversity is one of the most important evaluation criteria of ecosystems (Lavelle et al., 2006; Naeem et al., 2002; Schwartz et al., 2000), resistance to different forms of degradation (Schaefer & Schauermaun, 1990). In some ecosystems the local diversity of soil fauna may be more enormous, then the diversity of different groups of aboveground plants or animals (Schaefer & Schauermaun, 1990). Excessive reduction of the soil biodiversity, especially the loss of keystone species and/or species with unique functions may have some cascading ecological effects, which lead to the long-term deterioration of soil fertility and loss of agricultural productive capacity (Barrios, 2007; Huhta, 2007). Indices of soil invertebrates are the global indicators of soil quality and sustainability of ecosystems. The preservation of soil ecosystem services largely depends upon the preservation of soil invertebrates. Soil biodiversity also can have indirect effects to soil whether it functions as a carbon sink or source. Soil invertebrates present an important trophic level in the ecological chain nutrition of biocenosis. Invertebrates have a great importance for biological processes in soil,

increase the fertility and humus formation by mechanical decomposition of plant residues and formation of water-stable soil structure (Fragoso et al., 1997; Gilyarov & Striganova, 1987; Huhta, 2007; Jouquet et al., 2006).

In the Republic of Moldova the type of brown soils is formed under the European deciduous forests (beech, sessile oak, silver lime, hornbeam). Their formation is conditioned by the predominant altitudes (280-430 m), climatic conditions, age of geological deposits and they are spread on the highest hills of the Central Plateau of the Forests. These soils are considered as specific, because their surface does not exceed 20 thousand ha (Baltyanskiy, 1984). It was found that in the central forests, in the soil under deciduous forests (rock oak, beech), many species of invertebrates are widespread and typical burozems in the Carpathians and mountainous regions of Central and Southern Europe. The edaphic species of fauna, unique only to brown soils, especially from *Carabidae* and *Lumbricidae* fam. have been discovered (Gilyarov, 1965).

In this context, the *purpose of the research* was to investigate the abundance, biodiversity and ratio of trophic groups of invertebrates in brown soils of natural ecosystems and to determine the effect of the long-term agricultural land utilization of brown arable soils on the invertebrates' status for the biodiversity conservation and development of the national soil biota quality standards.

## MATERIALS AND METHODS

*Experimental sites* are located in the central zone of the Republic of Moldova, in the wooded steppe of the central - Moldovan forest province, in the district No. 8 of brown, gray forest soils and leached chernozems of the wooded steppe of hilly Kodru Forests. The plot with typical brown soil (profile 1 under forest; profile 2 under arable) is situated in the Tuzara village and Gorodische com., Kalarash region. The plot with luvic brown soil (profile 5 under forest; profile 6 under arable) is located in the Dolna com., Strasheni region.

Invertebrate' state in the virgin brown soil in the old-growth (primary) forest in the condition of natural ecosystems has been investigated in

comparison with the long-term arable brown soil in conditions of agricultural ecosystems.

*Status of invertebrates.* The state of invertebrates was identified from test cuts by manual sampling of soil layers to the depth of soil fauna occurrence with application of Gilyarov and Striganova's method (1987). The identification of invertebrate's diversity at the family's level and their classification according to nutrition were carried out by Gilyarov and Striganova's method (1987).

## RESULTS AND DISCUSSIONS

Brown soils are characterized by a low content of edaphic fauna (especially of the family of *Lumbricidae*) when conducting monitoring researches in 2020. There are many factors that have led to reduction in the number of invertebrates in the soil in this period. The main reasons are prolonged drought, low humidity and strong compaction of soil horizons, due to which the invertebrates have been migrated deeply or died. Meanwhile, significant differences were established between the abundance of biota in brown soils under forest and arable soils.

The number and biomass of the edaphic fauna in the typical brown soil under a forest are in 1.6 and 1.9 times higher than in the luvic brown soil under a forest (Table 1). However, the abundance of representatives of *Lumbricidae* family was higher in the luvic brown soil. It should be noted that moisture in the luvic brown soil was higher than in the typical brown soil in this period.

The biota status of brown arable soils is characterized by the significant reduction in the abundance and biomass of edaphic fauna in comparison with brown soils that was in conditions of natural ecosystems. Number of invertebrates and *Lumbricidae* fam. in the typical brown soil decreases on average from 72.0 to 21.3 ex m<sup>-2</sup> and from 2.7 to 0 ex m<sup>-2</sup>, biomass - from 12.0 to 1.6 g m<sup>-2</sup> and from 0.5 to 0 g m<sup>-2</sup>. Similar changes were observed in the luvic brown soil, where the number of invertebrates and *Lumbricidae* fam. decreases on average from 45.3 to 16.0 ex m<sup>-2</sup> and from 10.7 to 8.0 ex m<sup>-2</sup>, biomass - from 6.4 to 2.9 g m<sup>-2</sup> and from 4.3 to 2.1 g m<sup>-2</sup>.

The share of earthworms in the total abundance of invertebrates in the typical brown soil of natural ecosystems constitutes of 3.8% and their biomass - 4.2%. The average weight of one exemplar of *Lumbricidae* fam. in the virgin typical brown forest soil constitutes 0.19 g. The arable typical brown soil is characterized by a total lack of earthworms at the time of fauna sampling.

The share of *Lumbricidae* fam. in the total abundance of invertebrates in the luvic brown soil under the forest constitutes 23.6%, and the biomass - 67.2%. The average weight of a specimen of the *Lumbricidae* family in the luvic brown soil in conditions of natural ecosystems constitutes 0.40 g, and in conditions of agricultural ecosystems - 0.26 g. Thus, the weight of the earthworm in the arable soil has been significantly reduced.

Table 1. Number and biomass of invertebrates in typical brown soil under forest and arable land (n = 3 for each profile)

Soil	Land use	Profile	Number, ex m <sup>-2</sup>		Biomass, g m <sup>-2</sup>	
			total	<i>Lumbricidae</i> fam.	total	<i>Lumbricidae</i> fam.
Typical brown soil	forest	P1	72.0	2.7	12.0	0.5
	arable	P2	21.3	0	1.6	0
Luvic brown soil	forest	P5	45.3	10.7	6.4	4.3
	arable	P6	16.0	8.0	2.9	2.1

The base mass of fauna in brown soils under the forest is located in the 0-20 cm layer: in typical brown soil - 92.6%, in luvic brown soil - 76.6%. The number of invertebrates index decreases in the soil profile to a depth of 40 cm (Figures 1, 2).

Profile distribution of *Lumbricidae* fam. in the soils of natural ecosystems is uneven. The accumulation of *Lumbricidae* fam. in the typical brown soil was registered in the 10-20 cm layer (2.7 ex m<sup>-2</sup>). The largest number of earthworms in the luvic brown soil was found in the 20-40 cm layer.

The typical brown soil under arable does not contain earthworms. In the luvic brown soil in conditions of agricultural ecosystem fam. *Lumbricidae* (100%) is located in layers of 0-10 cm and 20-30 cm. Moreover, the luvic brown soil under the forest is characterized by the migration of *Lumbricidae* family into the underlying layers to a depth of 120-130 cm.

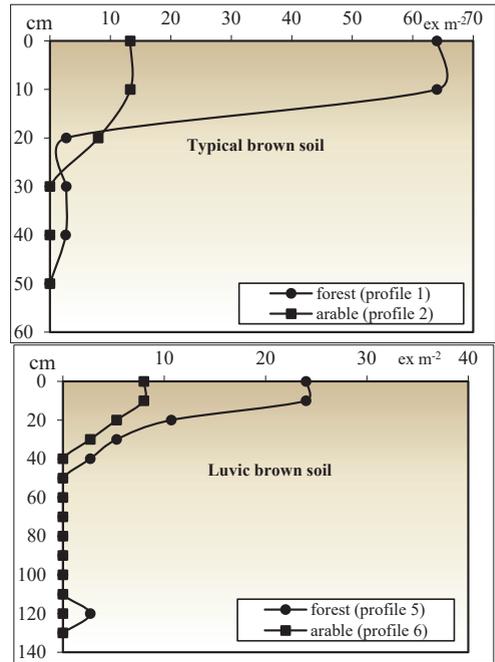


Figure 1. The profile distribution of invertebrates in the brown forest soils in natural and agricultural ecosystems (mean values, n = 3 for each soil layer)

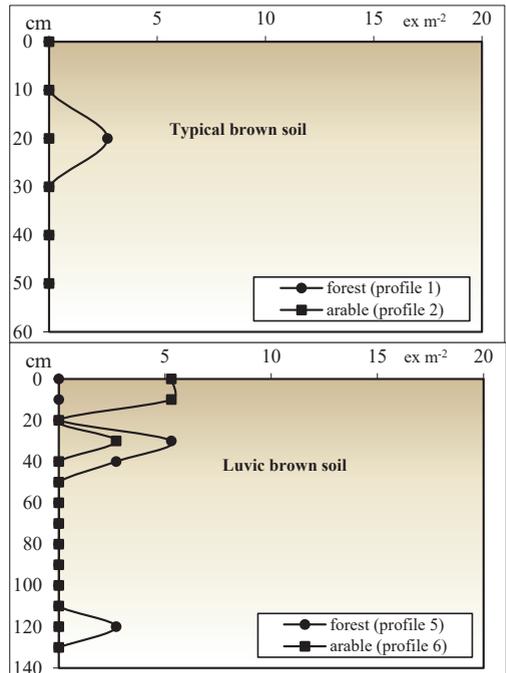


Figure 2. The profile distribution of invertebrates in the brown forest soils in natural and agricultural ecosystems (mean values, n = 3 for each soil layer)

Brown virgin soils are characterized by a high diversity of invertebrates compared to brown arable soils (Table 2). There are 10 families of invertebrates in the virgin typical brown soil. In addition to the *Lumbricidae* family the species from the families of *Clubionidae*, *Julidae*, *Hydromiidae*, *Carabidae*, *Coccinellidae*, *Scarabaeidae*, *Oniscidae*, *Geophilidae* and other have been found in the virgin typical brown soil. The abundant presence of the *Formicidae* family representatives is observed in virgin brown soils. *Formica rufa* is the typical representative of the *Formicidae* family. The number of anthills in the typical brown soil areals reaches about 200 units per hectare.

Edaphic fauna in the amount of 11 families has been identified in the luvic brown soil under forest. The species from the families of *Lumbricidae*, *Elateridae*, *Clubionidae*, *Julidae*, *Carabidae*, *Coccinellidae*, *Scarabaeidae*, *Oniscidae*, *Geophilidae*, *Chrysomilidae* and *Formicidae* have been found in the virgin luvic brown soil.

It should be noted that the biodiversity of edaphic fauna in both brown forest soils was similar.

The long-term use of plowing leads to the considerable decrease of the invertebrates' biodi-

versity. The typical brown soil in conditions of agricultural ecosystems contains 7 families of invertebrates, while the luvic brown soil - only 4 families of edaphic fauna. The species from the families of *Elateridae*, *Julidae*, *Scarabaeidae*, *Carabidae*, *Lasiocampidae*, *Reduviidae* and *Formicidae* were identified in the faunal samples from the arable typical brown soils. The arable luvic brown soil contains species of *Lumbricidae*, *Scarabaeidae*, *Carabidae* and *Formicidae* families.

Saprophagous predominate in the composition of the edaphic fauna in virgin brown forest soils. Their contribution to the total number of invertebrates is quite significant (Figure 3).

The share of saprophagous in the soil faunal complex in conditions of natural ecosystems constitutes 27.0% in the typical brown soil and 43.8% in the luvic brown soil respectively. The share of saprophagous in the total number of invertebrates in agroecosystems is 12.7% in the typical brown soil and 60.2% in the luvic brown soil.

The contribution of phytophagous in the total number of invertebrates is significantly lower and constitutes 23.0% in the typical brown soil and 25.1% in the luvic brown soil in natural ecosystems.

Table 2. Biodiversity of invertebrates (ex m<sup>-2</sup>) at the family's level in brown forest soils (mean values)

Invertebrates' families	Typical brown soil under forest (P1)	Typical brown soil under arable (P2)	Luvic brown soil under forest (P5)	Luvic brown soil under arable (P6)
<i>Lumbricidae</i>	2.7	0	10.7	8.0
<i>Elateridae</i> (larvae)	0	2.7	2.7	0
<i>Julidae</i>	10.7	2.7	2.6	0
<i>Scarabaeidae</i> (larvae)	5.3	5.3	2.7	2.7
<i>Carabidae</i> (imago+larvae)	10.7	5.3	2.6	2.6
<i>Coccinellidae</i>	8.0	0	2.7	0
<i>Geophilidae</i>	2.7	0	8.0	0
<i>Clubionidae</i>	13.2	0	2.6	0
<i>Hydromiidae</i>	10.7	0	0	0
<i>Oniscidae</i>	5.3	0	5.3	0
<i>Chrysomilidae</i>	0	0	2.7	0
<i>Lasiocampidae</i> (larvae)	0	2.7	0	0
<i>Reduviidae</i>	0	2.6	0	0
<i>Formicidae</i>	+++++	++	+	+
Unidentified species	2.7	0	2.7	2.7
<b>Total</b>	<b>72.0</b>	<b>21.3</b>	<b>45.3</b>	<b>16.0</b>

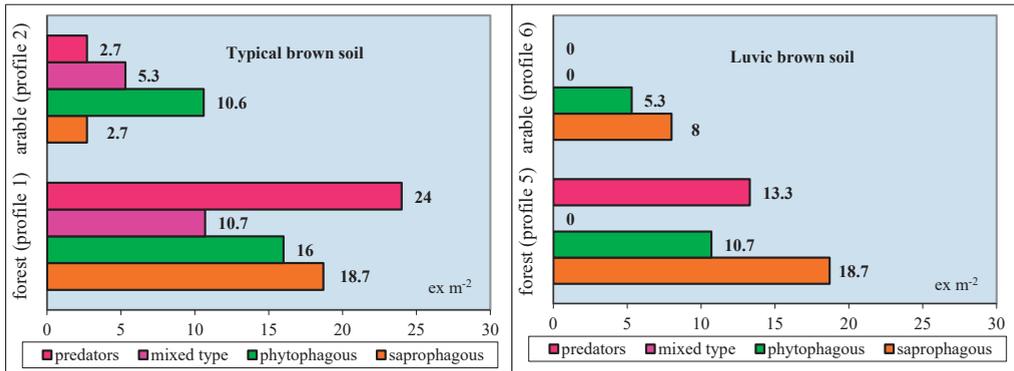


Figure 3. Composition of invertebrates according to the mode of nutrition in brown soils in natural and agricultural ecosystems (mean values, without *Formicidae* fam. and unidentified species)

The maximum number of phytophagous in percentage expression, registered in arable brown soils was the following: 49.8% in the typical brown soil and 39.8% in the luvic brown soil.

The content of invertebrates with the type of mixed nutrition in the typical brown soil under the forest was 15.4% and in conditions of arable - 24.9%. Invertebrates with mixed type of nutrition were not detected in the luvic brown soil.

The maximum number of predators in the amount of 31.1-34.6% was recorded in the brown soils under natural vegetation due to the high abundance of spiders. The contribution of predators in the total number of invertebrates in the typical arable brown soil was insignificant, the share constituted 12.6%. Predators in the arable luvic brown soil were absent.

Trophic pyramids in brown soils of natural ecosystems are characterized by a higher stability in comparison with arable brown forest soils. The quantitative relations between trophic levels of edaphic fauna of the typical brown soil are stronger in comparison to the luvic brown soil.

The long-term use of brown soils in agriculture leads to a decrease in the contribution of saprophagous and to a share growth of phytophagous in the total number of invertebrates (Table 3). The ratio between saprophagous and phytophagous decreased on the average by 4.5 times in the typical brown soil and by 15.9% in the luvic brown soil. These data indicate the dominance of phytophagous pests in the faunal complex of arable soils. Research results show that the

balance between invertebrate populations is disturbed, which over time leads to a decrease in the quality and fertility of arable soils.

Table 3. The ratio of trophic groups of invertebrates in brown soils

Profile	Soil, land use	Saprophagous/ Total	Saprophagous/ Phytophagous	Phytophagous/ Total
P1	Typical brown soil under forest	0.26	1.17	0.22
P2	Typical brown soil under arable	0.13	0.26	0.50
P5	Luvic brown soil under forest	0.41	1.75	0.24
P6	Luvic brown soil under arable	0.50	1.51	0.33

## CONCLUSIONS

The complex of invertebrates in brown soils of natural ecosystems is formed under the conditions of the share being he increased level of input and content of organic matter in the soil. 10-11 families of invertebrates were found in virgin brown soils. The edaphic fauna composition in natural ecosystems is complex and diverse. Saprophagous prevail in the composition of the edaphic fauna in the virgin brown soil under forest, accounting for 27.0% of the total abundance in the typical brown soil and 43.8% in the luvic brown soil, respectively. The relations between trophic levels are stronger in brown soils under forest, and trophic pyramids are more stable in the typical brown soil than in the luvic brown soil. A characteristic feature of the natural brown soils is the high concentration of invertebrates and

*Lumbricidae* family in the upper layers of soils. The base mass of fauna in the typical brown soil and in the luvisc brown soil under the forest is located in the 0-20 cm layer - 92.6% and 76.6%, respectively. Indicators of the edaphic fauna in soil profiles have been decreased with the depth. During the drought *Lumbricidae* fam. migrates to the underlying layers at depths >120 cm. Forests are habitat and natural medium for the conservation and restoration of the diversity and abundance of invertebrates in brown soils. The conservation of soil virgin standards in protected zones is important not only in terms of environmental protection, but also for significant scientific information.

The current state of the edaphic fauna in arable brown soils is characterized by a considerable decrease in the abundance, biomass and biodiversity compared to those of virgin brown soils in natural ecosystems. Dehumification processes, compaction and destruction of the soil structure as a result of the long-term agricultural use have worsened the habitat of the soil invertebrates. Furthermore, the soil doesn't obtain the plant residues sufficiently for the supply of edaphic fauna. The number and biomass of invertebrates in arable brown soils are lower by 2.8-3.4 times and by 2.2-7.5 times compared to brown soils of natural ecosystems. In arable soils only species from 4-7 families of edaphic fauna have been identified. There is a decrease in the number of saprophagous, especially in the *Lumbricidae* family, the destruction of levels and trophic links between invertebrates. The negative effects on the edaphic fauna were observed as a result of mineralization processes and long-term land management practices without organic fertilizers.

A land management with the fallow areas is recommended for the regeneration of soil invertebrates and the natural restoration of the quality of brown soils.

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## SOIL CHEMICAL INDICATORS OF MEADOW CHERNOZEM UNDER LONG-TERM ORGANIC FARMING: EXPERIENCE FROM UKRAINE

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### Abstract

*Organic farming as a system of sustainable crop production with maintaining soil fertility has an increasing interest because of its environmental benefits. Here we report changes of soil chemical indicators of virgin Meadow Chernozem after 8-years and 36-years of organic farming in Central Ukraine. We evaluate the effect of biological fertilizers application (manure, crop residues etc.) under reduced tillage (disking to 10-12 cm) on organic carbon and available nutrients content in 0-10 and 15-25 cm soil layers. Long-term organic farming increased N-NO<sub>3</sub> and N-NH<sub>4</sub> content in a soil and contributed to solubilization of phosphorus compounds. The decrease of soil organic carbon by 27% was found after 8-years of organic farming compared to initial content in topsoil; nevertheless biological fertilizers application during 36-years led to gradual replenishment of the soil organic carbon pool.*

**Key words:** biological fertilizers, soil organic carbon, available nutrients, soluble salts.

### INTRODUCTION

Organic agriculture is a sustainable alternative to conventional (chemical-based) agriculture using environmentally friendly strategies such as the application of biofertilizers and botanical and microbial products as pesticides (Durán-Lara et al., 2020). The global organic market is constantly growing, and this issue is very important for Ukraine as a well-known agricultural country which has a great potential in the production and consumption of organic products (Chyhryn et al., 2017).

Organic farming management relies on developing biological diversity to disrupt habitat for pest organisms, and the purposeful maintenance and replenishment of soil fertility as the main factor of high yields (Singh, 2021). Soil fertility has always been a primary focus and defining issue in organic agricultural systems (Heckman et al., 2009). Soil fertility management in organic farming needs a long-term integrated approach rather than the more short-term solutions to obtain a yield, which are common in conventional agriculture (Watson et al., 2002). The significant impact of long-term practicing of organic agriculture on the status

of various soil indicators was proved; organic farming contributes to the conservation of biological diversity (Singh, 2021) and increasing nutrients in the soil (Skrylnyk et al., 2019; Manjunatha et al., 2013) improves soil physical properties (reducing bulk density and increasing porosity and aeration) and chemical properties decreasing acidity (Singh et al., 2020).

Tillage has, and continues to be, widely used for weed control on organic farms globally, in spite of the deleterious impacts that tilling the soil can have on its ecosystem services (Carr, 2017). Many scientists concluded that reduced tillage, including No-till technology is a suitable method for increasing indicators of soil fertility in organic farming systems that may enhance the ecosystem services delivered by organic agriculture and make it more resilient to the effects of climate change (Gadermaier et al., 2012; Zikeli et al., 2017; Singh, 2021).

Organic agriculture with several advantages over the conventional system such as improvement of soil quality and crop quality, can overcome the environmental consequences of intensive farming (Abdelrahman et al., 2020). However, the organic system also has

several limitations that must be addressed, and proper management must be evaluated to promote the organic production system.

Nutrient pools in organically farmed soils are also essentially the same as in conventionally managed soils but in the absence of regular fertilizer inputs, nutrient reserves in less-available pools will be of greater significance (Shepherd et al., 2002). Organic production systems rely more on the use of on-farm inputs and less on external inputs, which reduce the input cost (Verma et al., 2020). Maeder et al. (2002) emphasized that enhanced soil fertility and higher biodiversity found in organic plots may render these systems less dependent on external inputs. On organically farmed soils, where the importation of materials to maintain soil fertility is restricted, it is important to achieve a balance between inputs and outputs of nutrients to ensure both short term productivity and long-term sustainability (Watson et al., 2002).

Organic farming systems provide the opportunity to deliver more soil ecosystem services than conventional practices. One such service could be soil organic carbon (SOC) accumulation (Blanco-Canqui, 2017). The Li et al. (2018) study demonstrates that fertilization strategies that include organic manure can increase the pool of stable carbon in the surface soil layer. Meanwhile, Hu et al. (2018), did not detect consistent differences in measured SOC between organically farmed soils and those that are managed conventionally, taking into consideration higher estimated carbon inputs in organic versus conventional systems. It has been shown that soil management under organic farming can enhance SOC, thereby mitigating atmospheric greenhouse gas increases, but until now, quantitative evaluations based on long-term experiments are scarce (Novara et al., 2019).

## MATERIALS AND METHODS

The field experiment was conducted at the Private Enterprise «Agroecology» Shyshaky district, Poltava region, Ukraine. Private Enterprise «Agroecology» have been practicing organic farming since 1982 and it is certified as an organic farm in accordance with the requirements of the standards of the EU

Council Regulation («EC 834/2007», «EC 889/2008»). The climate of the region is temperate continental with an accumulated temperature of 2500-2900°C and annual precipitation 480-536 mm. The soil - Meadow Chernozem with  $pH_{KCl}$  7.1, bulk density 1.05-1.1 g/cm<sup>3</sup>, and humus content 8.0% in topsoil (0-20 cm). At the beginning of the field experiment soil contained 11 mg/kg available nitrogen (N-NH<sub>4</sub> + N-NO<sub>3</sub>), 47-52 mg/kg available phosphorus (P<sub>2</sub>O<sub>5</sub>) and 100-110 mg/kg potassium (K<sub>2</sub>O). Crop rotation: winter wheat - sunflower - siderates (buckwheat+vetch, oats, wheat, alfalfa) - winter wheat - maize for silage - barley+esparcet - winter wheat. Crop rotations are designed with regard to maintenance of fertility with a focus on nutrient recycling. Reduced tillage was used (on the depth 10-12 cm). An average of 15-20 t ha<sup>-1</sup> of cattle manure or compost manure was applied annually. Soil samples were collected after crop rotation from the 0-10 cm and 15-25 cm soil layers.

Organic carbon content was determined by the Tyurin method. Humus content samples of 0.1 g of soil are taken from the samples and after a dichromate oxidation K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> with 10 ml the consumed oxidizer (oxidizing agent) is determined by titration with Fe(NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>6</sub>H<sub>2</sub>O using phenylanthranilic acid as an indicator.

Available nitrogen N (N-NH<sub>4</sub> + N-NO<sub>3</sub>) was determined by modification of the method of NSC ISSAR named after O.N. Sokolovsky: nitrogen extraction from the soil using potassium sulfate then nitrates determined photometrically with disulfophenol acid, and ammonium with Nessler's reagent. Available phosphorus and potassium was determined by the Chirikov method, extracting P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O by 0.5M acetic acid with a ratio of soil to a solution 1:25 followed by photometric determination. All measurements were performed in triplicate.

The ions content in water extract from the soil was determined by the following methods: ions of carbonates and bicarbonates – by titration with a solution of sulfuric acid to pH 8.3 (determination of carbonates) and pH 4.4 (determination of bicarbonates); chloride ions – by argentometric method according to Mohr; sulfate ion – by the volumetric method in the

presence of the indicator nitorochromazo; calcium and magnesium ions - by the complexometric method; sodium and potassium ions – by flame photometric method. The exchangeable cations content was estimated by complexometric method. Analysis of variance was performed using Statistica 10 software.

## RESULTS AND DISCUSSIONS

SOC dynamics are regulated by the complex interplay of climatic, edaphic and biotic conditions. The data presented in Figure 1 indicate that C-accumulation in Meadow Chernozem after long-term application of organic fertilizers and siderats under reduced tillage is greater in the 0-10 cm layer than in the 15-25 cm layer. After 8-year organic farming, Corg concentration decreased by 31% in the 0-10 cm soil layer and by 24 % in the 15-25 cm layer compared with the virgin soil. Meanwhile, organic carbon content in soil after 36 years of organic farming decreased by 28% and by 17% compared with the virgin soil in the 0-10 cm and 15-25 cm layers, respectively. SOC is considered as an integral parameter of soil fertility and a source of information for the diagnosis of soil degradation. Long-term organic farming contributes to sustaining organic carbon content in the soil at a fairly

high level (3.0-3.4%), but a slight decrease was found compared with virgin soil.

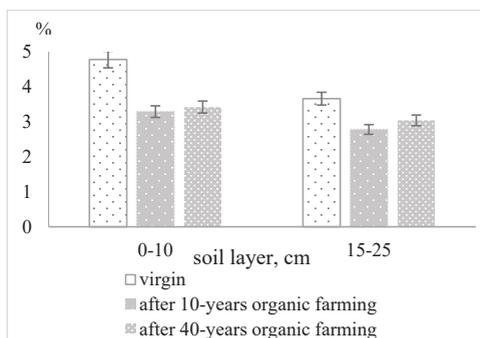


Figure 1. Corg content in the 0-10 cm and 15-25 cm layers of Meadow Chernozem under organic farming

Soil pH or soil reaction is an indication that plays a central role in many soil processes. Soil pH is an important predictor of bacterial community composition and diversity. Soil pH influences chemical properties and biological processes, including solubility, mobility, and availability of nutrients and trace metals. Results showed that organic farming during 36-years led to acidification of soil reaction: pH decreased by 0.4-0.5 units in 0-10 cm and by 1.5-1.7 units in 15-25 cm soil layer (Table 1). At the same time, an increased availability of nutrients in topsoil was observed.

Table 1. Effect of long-term organic farming on soil pH and available nutrients content in the 0-10 and 15-25 cm layers of Meadow Chernozem

Agricultural system	Soil layer, cm	Index				
		pHKCL	mg/kg			
			N-NH <sub>4</sub>	N-NO <sub>3</sub>	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O
No treatment (virgin soil)	0-10	5.7	12.0	1.8	67.8	144.6
	15-25	7.0	7.0	0.02	26.9	72.3
8-year organic farming	0-10	5.3	18.6	14.4	77.3	93.4
	15-25	5.5	8.1	6.1	73.9	81.3
36-year organic farming	0-10	5.2	12.5	7.6	93.3	96.4
	15-25	5.3	6.8	2.7	78.7	63.3
LSD <sub>0.05</sub>		0.2	2.6	2.2	7.5	8.8

<sup>1</sup>LSD 0.05 - Least Significant Difference at p=0.05

Nitrogen is a major nutrient, essential for all living organisms. Organic farming systems significantly increased the content of available nitrogen (N-NH<sub>4</sub> and N-NO<sub>3</sub>), especially in the upper soil layer (0-10 cm). Long-term application of organic fertilizers and siderats under reduced tillage caused an accumulation of significant amounts of nitrate nitrogen in

Meadow Chernozem. Siderats catch crops promote the sustainability of organic farming by nutrient uptake and transfer to the following main crops. This effect efficiently reduces the risk of nitrate leaching. Our results show that soil under 8-year organic farming is characterized by high NH<sub>4</sub>-N content, especially at a depth of 0-10 cm. But after 36

years of organic farming available N (N-NH<sub>4</sub> + N-NO<sub>3</sub>) were greatly reduced by 32-55% with the 8 years of organic farming. The application of organic farming during 36 years led to NH<sub>4</sub>-N increasing at a depth 0-10 cm by 0.5-6.6 mg/kg compared with the virgin soil, and at a depth 15-25 cm by 1.1 mg/kg. This may be attributed to the direct addition of these nutrients with organic fertilizers. The improved nitrogen status in the soil following organic fertilization can be explained by the high adsorption capacity of N and increased microbial activity that might have accelerated of mineralization during the growing season (Rutting et al., 2018). Biological nitrogen fixation by legume catch crops is an additional benefit in organic farming.

Phosphorus is the second most limiting nutrient in crop production. Phosphorus is abundant in soil however, the concentration of plant available P in the soil is generally low (Dhillon et al., 2017). The application of manure and compost with siderats under reduced tillage during 36 years led to accumulation of available phosphorus in the soil, presumably by increased the mineralization of organic P by microbial action. The highest P<sub>2</sub>O<sub>5</sub> content (77 and 93 mg/kg) were observed in the 0-10 cm soil layer after 8 and 36-years organic farming respectively. While, the highest accumulation of P<sub>2</sub>O<sub>5</sub> compared to virgin soil was found in the 15-25 cm layer, which is 2.7 times higher than in the same layer of virgin soil.

Potassium is an essential cation in all organisms that influences crop production and ecosystem stability (Srivastava et al., 2020). Available potassium loss in Meadow Chernozem was observed under long-term

organic farming. It was found that K<sub>2</sub>O content in 0-10 cm soil layer is by 50 mg/kg<sup>1</sup> less compared to the virgin soil. Even annual application of organic fertilizers (cattle manure or compost manure) during 36 years led to K<sub>2</sub>O loss form Meadow Chernozem compared to the virgin soil.

Exchangeable calcium cations (Ca<sup>2+</sup>) predominated in the studied soil samples: it ranged from 81.4 to 85% of the total cation content (Table 2). The content of Ca<sup>2+</sup>, which is essential for the structure of the soil and affects the humus accumulation, decreased after transition of virgin soil into arable one under organic farming. On plots of 36-years of organic farming and without treatment, the 0-10 soil layer is depleted in Ca<sup>2+</sup> compared to 15-25 cm. On a plot that have been engaged in organic farming just for 8 years, this relationship is inverse – the lower soil layer, the less content of exchangeable calcium cations (Ca<sup>2+</sup> in 15-25 cm soil layer was by 8.6 % less than in the 0-10 cm layer). For magnesium (Mg<sup>2+</sup>), this difference was even bigger and amounted up to 29.7 %. Total content of exchangeable Ca<sup>2+</sup> and Mg<sup>2+</sup> was the greatest in virgin soil, meanwhile on plot of 8-years of organic farming the sum of exchangeable cations was approximately in 2 times less 15-25 cm layer than in virgin soil. The use of land in agricultural production increases the sodium (Na<sup>+</sup>) content in the 0-10 cm layer by 0.9-1.5 mmol/kg soil, which is not a significant change. In general, the content and composition of exchangeable cations is most favorable in the soil under 36-years of organic farming.

Table 2. Effect of long-term organic farming on exchangeable cations in the 0-10 and 15-25 cm layers of Meadow Chernozem

Agricultural system	Depth of sampling, cm	Content in soil, mmol kg <sup>-1</sup>				
		Ca <sup>2+</sup>	Mg <sup>2+</sup>	Na <sup>+</sup>	K <sup>+</sup>	∑ cations
No treatment (virgin soil)	0-10	203.7	30.3	0.9	4.6	239.5
	15-25	331.5	65.5	1.1	2.6	400.7
8-years of organic farming	0-10	184.2	32.3	2.4	3.5	222.4
	15-25	168.3	22.7	1.1	3.3	195.4
36-years of organic farming	0-10	198.7	39.6	1.8	3.8	243.9
	15-25	228.9	32.8	2.4	2.9	267.0
LSD <sub>0.05</sub>		8.2	2.4	0.5	1.5	

<sup>1</sup>LSD 0.05 - Least Significant Difference at p=0.05

For Meadow Chernozems the salt composition is of great importance, since they are formed with the participation of groundwater, which can have different degrees of mineralization. The examined soil samples contained small

quantities of water-soluble salts. In virgin soil, salts presented by bicarbonates and chlorides of calcium and magnesium, mainly in the 15-25 cm layer (Table 3).

Table 3. Effect of long-term organic farming on salt composition in the 0-10 and 15-25 cm layers of Meadow Chernozem

Agricultural system	Depth of sampling, cm	pH <sub>H2O</sub>	Content in water solution, meq/kg							
			HCO <sub>3</sub> <sup>-</sup>	Cl <sup>-</sup>	SO <sub>4</sub> <sup>2-</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Na <sup>+</sup>	K <sup>+</sup>	∑ eq cations
No treatment (virgin soil)	0-10	6.7	2.8	1.1	1.4	3.5	1.3	0.3	0.2	5.3
	15-25	8.1	7.5	1.5	0.9	5.7	3.5	0.6	0.1	9.9
8-years of organic farming	0-10	6.4	1.5	1.1	2.8	2.4	1.6	1.2	0.2	5.4
	15-25	6.6	1.8	0.4	1.8	1.9	1.4	0.5	0.2	4.0
36-years of organic farming	0-10	6.4	2.0	0.4	2.6	2.6	1.4	0.8	0.2	5.0
	15-25	6.7	2.3	0.9	1.2	1.9	1.4	1.0	0.1	4.4
LSD <sub>0.05</sub>			0.7	0.4	0.8	1.0	0.3	0.2	0.1	

<sup>1</sup>LSD 0.05 - Least Significant Difference at p=0.05

As a result of long-term organic farming the content of bicarbonates and chlorides decreased by 28.6-46.4% (0-10 cm) and by 69.3-79% (in 15-25 cm) compared to the virgin soil. At the same time, the content of sulfate anions (SO<sub>4</sub><sup>2-</sup>) increased by 2 times, amounting up to 2.6-2.8 meq/kg (0-10 cm) and 1.2-1.8 meq/kg (15-25 cm). The content of the calcium cation (Ca<sup>2+</sup>) in the soil solution decreased by 25.7-31.4 % in the upper layer and especially strongly in the lower layer (by 66.7%).

## CONCLUSIONS

Findings indicate that transition from virgin soil to arable soil with following long-term organic farming with annual application of organic inputs, using siderats and reduced tillage results in significant change of soil chemical indicators, which are critical for long-term fertility maintenance. After 36-year organic farming soil agrochemical characteristics changed as follows: pH decreased by 1.05 units, available nitrogen (N-NH<sub>4</sub> + N-NO<sub>3</sub>) increased by 4.4 mg/kg, available phosphorus (P<sub>2</sub>O<sub>5</sub>) increased by 38.7 mg/kg, meanwhile potassium decreased by 28.6 mg/kg. While Meadow Chernozem under long-term organic farming has larger pools of nutrients compared with the virgin soil, but SOC loss was observed after the transition of

virgin soil to arable one with a positive trend of organic carbon accumulation according to the term of organic farming.

The involving of soil into organic farming agricultural system leads to a change in content of exchangeable bases in Meadow Chernozem. The content of exchangeable cations, except Na<sup>+</sup>, tends to decrease under long-term organic farming. Organic farming did not induce accumulation of salt in the soil arable layer, but a significant decrease of water-soluble Ca<sup>2+</sup> and simultaneous increase Na<sup>+</sup> were observed. The composition of the salts of Meadow Chernozem is bicarbonates and chlorides of calcium and magnesium, which has low toxicity to plants. Nevertheless, a necessary measure to preserve soil fertility during the agricultural use of Meadow Chernozem is a regular monitoring of the salts content and absorbed cations.

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## ESTIMATION OF MEASUREMENT UNCERTAINTY FOR PHOSPHORUS SPECTROPHOTOMETRIC DETERMINATION IN ORGANO-MINERAL FERTILIZERS

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### Abstract

*This study evaluates the measurement uncertainty of total phosphorus determinations in organo-mineral fertilizers. Total phosphorus was determined by UV-VIS spectrophotometry using an in-house ammonium molybdate and ammonium metavanadate method. The main parameters controlling the result of the analysis were identified and combined uncertainty ( $U_c$ ) was calculated. The expanded uncertainty ( $U$ ) of the method, obtained by multiplying the combined uncertainty by the coverage factor  $k = 2$  (confidence level 95%) was 6.43%. It was noticed, that the main sources of uncertainty were: type A (i.e. repeatability) and type B (i.e. uncertainties related to the analytical balance, volumetric glassware, stock solution and spectrophotometer).*

**Key words:** measurement uncertainty, phosphorus, spectrophotometry, organo-mineral fertilizers.

### INTRODUCTION

As the development of agricultural productivity is directly related to the use of fertilizers, it is necessary to analyze them with sensitive techniques in order to monitor their quality. Common fertilizers are either of inorganic or organic composition, of biological or chemical nature (Cox et al., 2003; Viso & Zachariadis, 2018).

Most common mineral fertilizers contain three primary macronutrients that play an important role in plant development: nitrogen (N), phosphorus (P), and potassium (K). Fertilizers may also contain lower levels of the three secondary nutrients, calcium (Ca), sulfur (S), magnesium (Mg), as well as micronutrients, such as: boron (B), manganese (Mn), iron (Fe), zinc (Zn), copper (Cu), and molybdenum (Mo) (Pelizzaro et al., 2019). In addition, organo-mineral fertilizers contain materials of biological origin (i.e. animal wastes, agricultural/crop residues, compost, biosolids etc.). Therefore, organo-mineral fertilizers are defined as mixtures of organic and mineral fractions that can be produced in several N, P and K proportions suitable for crop requirements. The use of organic waste in

agriculture represents an economical and environmental viable practice mainly because it allows the recovery of several chemical elements, such as N, P, K and microelements (Crusciol et al., 2020).

The quantitative determination of these elements in fertilizers is important for product quality control and regulatory requirements. Fertilizers that are produced or sold within the European Union (EU) must comply with the concentration limits specified in Regulation EC 2003/2003 related to mineral products. In addition, many EU member states have detailed, national rules and standards in place for non-harmonized fertilizers that do not apply to EC-fertilizers. Italy, for example, has regulations that relate to organic fertilizers. In the US, the Environmental Protection Agency sets standards and regulations for some types of fertilizers but States can adopt regulations that are more stringent and/or broader than the Federal regulations. In China, fertilizers are subject to approval by the Ministry of Agriculture in compliance with the Food Safety Law (*Regulation (EC) No. 2003/2003*, 2003; Pelizzaro et al., 2019).

Given the impact of chemical analysis and quality control of fertilizers on agricultural

production, several techniques have been reported in the literature for the determination of these elements in fertilizers (Viso & Zachariadis, 2018; Pelizzaro et al., 2019). Analysis of extracted P in fertilizers is typically performed using (i) gravimetric; (ii) volumetric; and (iii) colorimetric testing methods. More recently, inductively coupled plasma atomic emission spectroscopy (ICP-AES) has been used for the analysis (Viso & Zachariadis, 2018; Pelizzaro et al., 2019). For total phosphorus estimation, the gravimetric quinoline phosphomolybdate method is generally preferred because of the minimal interference of other ions and its accuracy and simplicity (Pelizzaro et al., 2019). Another common method providing acceptable accuracy and simplicity is the spectrophotometric vanadate-molybdate assay (Motsara & Roy 2008).

All measurements are affected by a certain error. The measurement uncertainty gives information on the size of the measurement error. Therefore, the measurement uncertainty is an important part of the reported result (Magnusson et al., 2017).

Many important decisions are based on the results of chemical quantitative analysis; the results are used, for example, to estimate yields, to check materials against specifications or legal (allowable) limits, or to estimate monetary value (Magnusson et al., 2017). Whenever decisions are based on analytical results, it is important to have an indication of the quality of the results. One useful measure used to demonstrate the quality of the results is measurement uncertainty (Eurachem, 2012; Magnusson et al., 2017). The data user needs it together with the result to make a correct decision. Also, the laboratory needs it to verify its' own quality of measurement given that estimation of the measurement uncertainty is required by ISO 17025 (Magnusson et al., 2017; Romanian Standards Association, 2018). According to the Eurachem/CITAC Guide (2012) the measurement uncertainty is a parameter associated with the result of a measurement that characterises the dispersion of the values that could reasonably be attributed to the measurand. There are general rules for evaluating and expressing uncertainty for a wide range of measurements (Barwick &

Ellison, 2000; Eurachem, 2012). The international guidelines require the identification of all possible sources of uncertainty associated with the procedure; the estimation of their magnitude from either experimental or published data (quality control charts, validation, proficiency testing, certified reference materials etc.); and the combination of these individual uncertainties to give standard and expanded uncertainties of the procedure (Cox et al., 2003; Barwick & Ellison, 2000; Vetter, 2001; Eurachem, 2012; Ionescu et al., 2014; Magnusson et al., 2017; Romanian Standards Association, 2018).

This paper is focused on estimation the measurement uncertainty related to total phosphorus determination by UV-VIS spectrophotometry in organo-mineral fertilizer samples.

## MATERIALS AND METHODS

### *Reagents and solutions*

For the preparation of the working standard solutions and acid digestion of fertilizer samples Certipur Phosphorus 1000 mg/L standard,  $\text{NH}_4\text{VO}_3$ ,  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$ ,  $\text{HNO}_3$  (65%),  $\text{HCl}$  (37%), and  $\text{H}_2\text{SO}_4$  (95-97%) of analytical grade were obtained from Merck (Germany). All solutions were prepared using distilled water.

Ammonium molybdate solution (5%) was prepared by dissolving 50 g of  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$  in 500 mL distilled water. The final volume of the solution was adjusted to 1000 mL. Ammonium vanadate solution (0.25%) was obtained by dissolving 2.5 g of  $\text{NH}_4\text{VO}_3$  in 500 mL distilled water and adding 20 ml concentrated nitric acid. Finally, the solution was diluted to 1000 mL (Romanian Standards Association, 1998).

An interlaboratory comparisons organo-mineral sample (ILC) obtained from Bipea (France) was used as secondary/external reference material in the experimental trials.

All weighing operations were carried out using METTLER TOLEDO AG204 analytical balance. Acid digestion was performed by using a simple heated sand bath. The absorbance of the sample solutions was measured with a UV-VIS spectrophotometer (CECIL Instruments).

### Preparation of Working Standard Solutions

Working standard solutions containing 0.00, 0.005, 0.01, 0.02, 0.03, 0.04, 0.05 mg/mL were prepared by proper dilutions of the 1000 mg/L phosphorus standard (5 mL hydrochloric acid was added to each standard).

The calibration curve was prepared by pipetting 10 mL of the above mentioned standard solutions in 25 mL Erlenmeyer flasks and addition of 2 mL of each colorimetric reagent (5% molybdate solution and 0.25% vanadate solution). These solutions contain 0.00, 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 mg of phosphorus.

After 30 min, the color development is complete and the absorbance can be measured at 470 nm.

### Method description

The measuring principle is based on the photometric yellow method (molybdate-vanadate), which has been used for years to measure orthophosphate in mineral fertilizers (Romanian Standards Association, 1998).

The flow sheet for total phosphorus extraction and determination in organo-mineral fertilizers is illustrated in Figure 1.

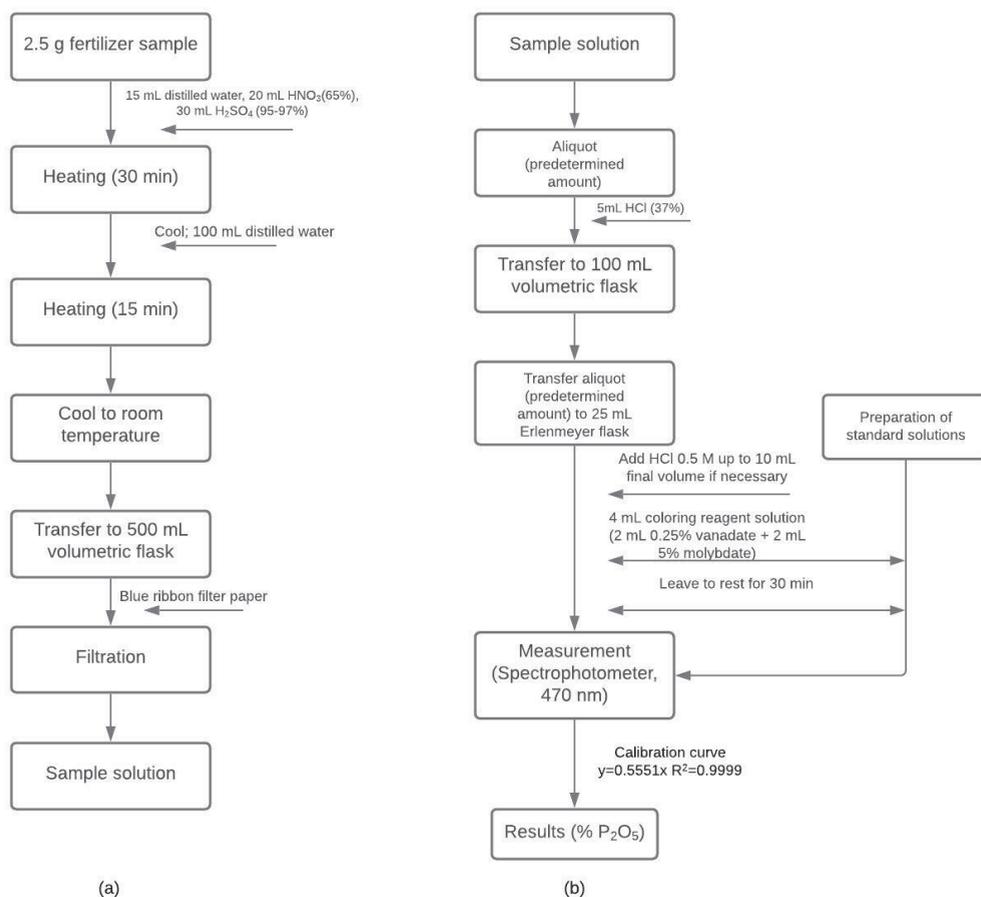


Figure 1. Flow sheet for total phosphorus extraction (a) and determination (b) in organo-mineral fertilizers

## RESULTS AND DISCUSSIONS

The measurement uncertainty was calculated by following an internal procedure and international agreed approaches (Eurachem, 2012). The evaluation of measurement

uncertainty of total phosphorus determination consisted the following steps: specification of the measurand, identification of uncertainty sources, quantification of uncertainty components and calculation of combined and expanded uncertainty.

## Specification of the measurand

$$\text{Phosphorus (\% } P_2O_5) = \frac{c \times r_1 \times r_2 \times 100}{m \times 1000} \quad (1)$$

where:

$c$  - concentration of phosphorus corresponding to the absorbance read on the calibration curve (mg);

$r_1$  - the ratio between the initial volume ( $v_1$ ) of the sample solution and aliquot ( $a_1$ );

$r_2$  - the ratio between the volume ( $v_2$ ) at which the aliquot 1 ( $a_1$ ) was transferred and the volume used for colorimetry ( $a_2$ );

$m$  - mass of the sample (g).

## Identification of uncertainty sources

Figure 2 illustrates the cause and effect diagram for the standard uncertainty of the method.

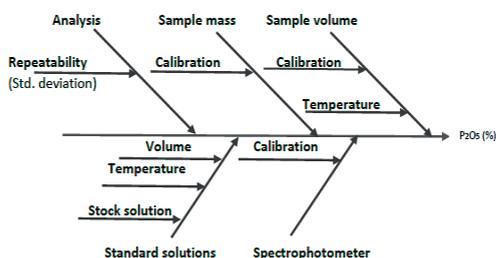


Figure 2. Cause and effect diagram for total phosphorus determination

As schematized in Figure 2, the following sources of were identified: (a) uncertainty from the standard and standard dilution to obtain the working calibration solutions; (b) uncertainty from the spectrophotometer; (c) the dilution factor includes the contributions of uncertainties from the volumetric flasks and pipettes and the volume expansion at the temperature of the laboratory; (d) uncertainty of the sample mass / analytical balance (obtained from the calibration certificate); (e) repeatability, evaluated by measuring 10 sample replicates of the analyte. The measurements were conducted by the same analyst using the same UV-VIS instrument under the same working conditions.

## Quantification of individual components

### Repeatability

The characteristics of the ILC sample used as secondary reference material in the experiments are given in Table 1.

Table 1. Characteristics of the ILC sample used as secondary reference material

Statistical parameter	% total phosphorus
Assigned value, $x_{pt}$	3.39
Standard uncertainty of the assigned value, $u(x_{pt})$	0.06
Robust standard deviation of the results, $s(x_{pt})$	0.30
Tolerance value, VT	0.80
Maximum value	4.19
Minimum value	2.59

All steps of the measurement procedure (Figure 1) were included in the uncertainty associated with the repeatability of the overall experiment. Repeatability data is given in Table 2.

Table 2. Repeatability of the spectrophotometric method for determination of phosphorus in ILC sample

Sample	% total phosphorus
Replicate 1	3.54
Replicate 2	3.60
Replicate 3	3.64
Replicate 4	3.54
Replicate 5	3.64
Replicate 6	3.40
Replicate 7	3.54
Replicate 8	3.60
Replicate 9	3.64
Replicate 10	3.35
Statistical parameter	% phosphorus
Average value	3.55
Standard deviation, $n = 10$	0.11

### Sample mass, dilution factor and spectrophotometer contribution

The uncertainty related to the calibration of all equipment was evaluated using the data from the calibration certificates. The standard uncertainty associated with the mass of the sample was calculated (Eq. 2), using the data from the calibration certificate (i.e. linearity and eccentricity) (De Oliveira, 2016). This contribution was counted twice, once for the tare and once for the gross weight, because each is an independent observation and the linearity effects are not correlated (Eurachem, 2012). Standard uncertainties were calculated according to the following equations:

$$u(m) = \sqrt{\left(\frac{U_{eccentricity}}{2}\right)^2 + \left(\frac{U_{linearity}}{2}\right)^2} \quad (2)$$

$$u(v) = \sqrt{\left(\frac{U_{\text{calibration}}}{2}\right)^2 + \left(\frac{V_{\text{temp}}}{\sqrt{3}}\right)^2} \quad (3)$$

$$u(x) = \frac{U_{\text{calibration}}}{2} \quad (4)$$

For all volumetric equipment the uncertainty consists of 2 components: calibration uncertainty and uncertainty due to the volume expansion at laboratory temperature ( $20 \pm 4^\circ\text{C}$ ), calculated by means of Eq. 5 (Droic & Ros, 2002; Eurachem, 2012):

$$V_{\text{temp}} = V_{\text{calibration}} \times Dt \times 2.1 \times 10^{-4} \quad (5)$$

where:

$V_{\text{temp}}$  - uncertainty due to the volume expansion at laboratory temperature;

$V_{\text{calibration}}$  - volumetric glassware uncertainty according to calibration certificate;

$2.1 \times 10^{-4} \text{ } ^\circ\text{C}^{-1}$  - coefficient of volume expansion for water;

$Dt$  - temperature variation in the laboratory ( $20 \pm 4^\circ\text{C}$ ).

Standard uncertainty due to sample dilution was estimated by summing the uncertainties of the volumetric glassware to the volume expansion (Table 3). The relative uncertainties due the dilution factor of the sample (volumetric flasks+aliquots) were combined

into one contribution for the overall experiment. Standard uncertainty related to the spectrophotometer was calculated by means of Eq. (4).

*Working standard solutions and stock solution contribution*

An example for the preparation of one working standard solution is summarized in Table 4. The same procedure was repeated for all standards. Standard uncertainty associated to the stock solution was estimated by means of Eq. 4 (Table 4). The relative uncertainty related to the dilution of the standards was obtained by the sum of the relative uncertainties due to the dilution factor and stock solution.

The relative contribution of each component, as well as the combined and expanded uncertainties are summarized in Table 5. The combined standard uncertainty of the method was estimated according to Eq. (6). The expanded uncertainty (U) was obtained by multiplying the combined uncertainty with a coverage factor of 2 (Table 5).

$$u(P_2O_5) = \bar{x} \sqrt{\left(\frac{u(r)}{\bar{x}}\right)^2 + \left(\frac{u(c)}{c}\right)^2 + \left(\frac{u(m)}{m}\right)^2 + \left(\frac{u(v)}{v}\right)^2 + \left(\frac{u(au)}{au}\right)^2} \quad (6)$$

Table 3. Standard uncertainties related to sample mass, dilution factor and spectrophotometer

Source/ Quantity	Uncertainty components		Distribution		Eq./us
<b>Analytical balance</b>					
Mass of the sample	Eccentricity	Linearity	normal	normal	Eq. (2)
2.5 g	0.0005 g	0.0002 g	2	2	0.0004 g
<b>Dilution factor (volume)</b>					
Volumetric flask (v1)	Calibration	Volume expansion	normal	rectangular	Eq. (3)
500 mL	0.05 mL	0.2425 mL	2	$\sqrt{3}$	0.1422 mL
Volumetric flask (v2)	Calibration	Volume expansion	normal	rectangular	Eq. (3)
100 mL	0.03 mL	0.0485 mL	2	$\sqrt{3}$	0.0318 mL
Aliquot 1	Calibration	Volume expansion	normal	rectangular	Eq. (3)
10 mL	0.023 mL	0.0048 mL	2	$\sqrt{3}$	0.0118 mL
Aliquot 2	Calibration	Volume expansion	normal	rectangular	Eq. (3)
10 mL	0.023 mL	0.0048 mL	2	$\sqrt{3}$	0.0118 mL
Spectrophotometer	Calibration		normal		Eq. (4)
0.225 AU	0.005 AU		2		0.0025 AU

Table 4. Standard uncertainties related to the preparation of working standard solutions and to the stock standard solution

Source/Quantity	Uncertainty components		Distribution		Eq./us
<b>Dilution factor (volume)<sup>i</sup></b>					
Burette 1 mL	Calibration 0.015 mL	Volume expansion 0.00049 mL	normal 2	rectangular $\sqrt{3}$	Eq. (3) 0.0075 mL
Volumetric flask 100 mL	Calibration 0.03 mL	Volume expansion 0.0485 mL	normal 2	rectangular $\sqrt{3}$	Eq. (3) 0.0318 mL
<b>Stock solution<sup>ii</sup></b>					
Concentration 1004 mg/kg	Calibration 6 mg/kg		normal 2		Eq. (4) 3 mg/kg

<sup>i</sup> Standard uncertainty related to the preparation of one working standard solution

<sup>ii</sup> Standard uncertainty related to the stock standard solution

Table 5. Expanded and combined uncertainties of the method for phosphorus determination in organo-mineral fertilizer by UV VIS spectrophotometry

Uncertainty component	Value	Unit	Type
Phosphorus content mean ( $\bar{x}$ )	3.55	% P <sub>2</sub> O <sub>5</sub>	A
Relative standard uncertainty of the sample repeatability $u(r)/\bar{x}$	0.0285	-	A
Relative standard uncertainty of the calibration solutions $u(c)/c$	0.0096	-	A&B
Relative standard uncertainty of the sample mass $u(m)/m$	0.0002	-	B
Relative standard uncertainty of the dilution factor of the sample $u(v)/v$	0.0017	-	B
Relative standard uncertainty of the spectrophotometer $u(au)/au$	0.0111	-	B
Combined standard uncertainty ( $u_c$ )	0.1140	% P <sub>2</sub> O <sub>5</sub>	A+B
Expanded uncertainty ( $U_c = (u_c \times 100)/\bar{x}$ )	3.21	%	
Expanded uncertainty, $k=2$ ( $U = U_c \times 2$ )	6.43	%	

### Uncertainty budget

The uncertainty budget as relative contributions of the different components are shown in Figure 3. As shown in Figure 3, the main sources of uncertainty of the result of measurement were identified as contributions

from repeatability, standard solutions preparation and spectrophotometer, while the contribution of the sample mass/ analytical balance has no influence on the overall uncertainty.

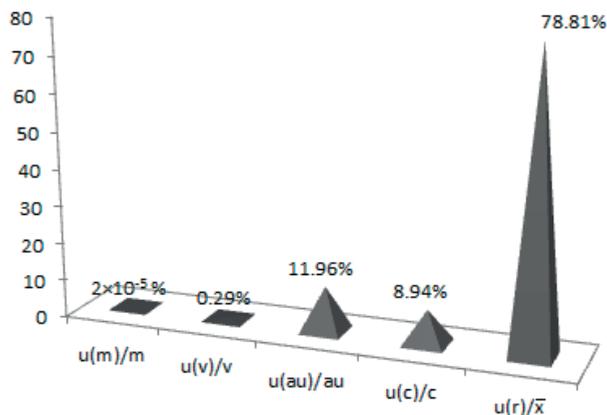


Figure 3. The uncertainty contributions as relative uncertainties ( $u(m)/m$ : uncertainty related to sample mass uncertainty;  $u(v)/v$ : uncertainty related to sample dilution;  $u(au)/au$ : spectrophotometer uncertainty;  $u(c)/c$ : uncertainty associated to the standard solutions;  $u(r)/\bar{x}$  average: uncertainty associated to the repeatability)

## CONCLUSIONS

This study evaluated the measurement uncertainty of the result of total phosphorus determinations in organo-mineral fertilizers.

The identified uncertainty components were the repeatability, standard solution dilutions, spectrophotometer, sample mass (analytical balance) and sample dilution factor. It was observed that the largest contribution comes from the analysis process (i.e. repeatability, 78.8%).

The result of measurement and the expanded uncertainty estimated by using Eurachem guidelines was  $3.55 \pm 0.23 \% \text{P}_2\text{O}_5$ .

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## DISPERSION AND REGRESSION ANALYSIS ON GRAIN YIELD AND NITROGEN FERTILIZATION OF TRITICALE VARIETIES II

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### Abstract

*The aim of the study is to analyze the responsiveness of two Bulgarian varieties of triticale to the rate of nitrogen fertilization in the formation of grain yield. The study area was Field Crops Institute-Bulgaria. The test period concluded 2015/2017. Were tested two varieties triticale - Attila and Boomerang. Were included in the experimental production tree rates of nitrogen fertilizer (kg/ha) -  $N_{60}$ ,  $N_{120}$  and  $N_{180}$ , incorporated tillering phase. The phosphorus fertilizer was 60 kg/ha, incorporated in autumn. Dispersion and regression analysis was applied to establish statistically significant influences of the studied factor and differences between the tested variants. Mineral fertilization at rates of 60, 120 and 180 kg N/ha had a confirmed statistical effect. Only the difference between the control variant and  $N_{60}$  had a proven effect. For both tested varieties, the largest increase in grain yield compared to the theoretical yield can be expected when fertilizing with 60 kg N/ha.*

**Key words:** grain yield, nitrogen fertilization, statistical analysis, triticale.

### INTRODUCTION

Statistical analyzes are widely used in biological research. The main task of statistical analyzes is to establish quantitative relationships of a statistical nature between the observed quantities. The statistical dependences between the traits, apart from their strength and direction, can also be analyzed by nature, by quantitative ratios between the comparative traits with the help of regression analysis. Regression analysis is preferred to get the relationship between independent variables (or inputs) and dependent variable (or output) (Jiang and Liao, 2020). In simple liner regression, the value of one variable (x) is used to predict the value of the other variable (y) by means of a simple mathematical function, the linear regression equation, while quantifies the straight-line relationship between the two variables (Pandey, 2020). By establishing this connection, farmers can predict crop yields by managing investments worthwhile, as economic modeling of crop production is complex in the agricultural sector (Amoozad-Khalili et al., 2020). The three most commonly required and widely used nutrients in agriculture are nitrogen (N), phosphorus (P) and potassium (K) (Dhillon et al., 2019). Synthetic fertilizers help increase crop

productivity. However, a significant amount of N application is lost to the environment through nitrification, denitrification, leaching and evaporation (Cao, Lu & Yu, 2017). As a result of the statistical analysis, more or less generalized objective values are obtained, which quantitatively reflect individual characteristic features of the statistical regularities and aggregates.

Analysis of variance is part of the statistics studying the influence of one or more grouping variables on a quantitative one. As in regression analysis, it is customary to call this dependent variable a response. Predictors, however, are called factors here. At the heart of the analysis of variance is the possibility that the sum of the squares of the response deviations (SSY) can be decomposed into several independent sums of squares, thus making it possible to test different hypotheses about the influence of factors on the response. So far triticale is the only cereal created artificially. The name is derived from the merging of the Latin names triticum (wheat) and secale (rye) (Bonchev, 2020). Triticale is cultivated on a global scale with the main production areas in Central and Eastern Europe, where the largest producers are Poland, Germany, France, Belarus, and Russia (FAO, 2015).

The aim of the study is to analyze the responsiveness of two Bulgarian varieties of triticale to the rate of nitrogen fertilization in the formation of grain yield.

## MATERIALS AND METHODS

The study area was Field Crops Institute-Chirpan, Bulgaria (42°11'58"N, 25°19'27" E). The test period was between 2015 and 2017. The experimental plot was 12 m<sup>2</sup> in four replications. Two varieties of triticale were tested – Attila and Boomerang with sunflower predecessor. Three rates of nitrogen fertilizer (kg/ha) – N<sub>0</sub>, N<sub>120</sub> and N<sub>180</sub>, were included in the experimental production, incorporated during the tillering phase. The phosphorus fertilizer was 60 kg/ha, incorporated in autumn. As a control option was adopted N<sub>0</sub>P<sub>0</sub>.

Analysis of variance (ANOVA) was applied in order to determine the statistically significant effects of the studied factors and differences (LSD) between the tested variants, analysis of variance (ANOVA) was applied. The following model was applied:

$$Y_i = \mu + ai + ei$$

where:  $Y_i$  are the meaning and number of the dependent and factor variables;  $\mu$  are the average of the test results;  $ai$  is the effect of factors;  $ei$  is random error.

Data regression was determined with the software Statistica 13.0 (TIBCO, Software, 2018). The following model of regression dependence equation was used:

$$y = a + bx,$$

where:  $y$  are the values of the dependent variable or function (in our case grain yield);  $x$  are the values of the independent variable or argument (in our case fertilization rates);  $a$  is the parameter (coefficient) reflecting the distance from the zero point of the coordinate system to the beginning of the regression line;  $b$  is the angular coefficient characterizing the slope of the regression line.

## RESULTS AND DISCUSSIONS

The data presented in Table 1 shows that mineral fertilization has a stronger effect in the Attila variety than in the Boomerang variety in the formation of grain yield (GY). Fertilization

in the Attila variety leads to an increase in the statistically significant effect with increasing fertilizer rate. In the case of the Boomerang variety, the N<sub>120</sub> and N<sub>180</sub> norms have the same proven effect (P = 1%). Janašauskaite (2013) also reports that nitrogen fertilization has a proven significant effect on GY.

Table 1. GY variance analysis on average for the study period (2015/2017)

Fertilization rates	Attila	Boomerang
	relative to the variant without fertilization	relative to the variant without fertilization
N <sub>0</sub>	-	-
N <sub>60</sub>	*	*
N <sub>120</sub>	**	**
N <sub>180</sub>	***	**

ns: no significant; \*, \*\*, \*\*\* significant at P = 5%, P = 1% and P = 0.1%

The analysis of variance presented in Table 2 confirmed the results in Table 1, where it can be seen that the effect of fertilization had a stronger effect for the Attila variety than for the Boomerang variety, 73.2% and 56.6% of the total variation, respectively. From these results it can be assumed that the Attila variety is more strongly influenced by mineral fertilization. This is confirmed by Stoyanov (2020), who reports that obtaining high yields from the cultural plant is related to the growing of cultivars, which are adaptable to certain soil and climatic conditions.

Table 2. Dispersion analysis of GY average for the study period (2015/2017)

Varities	Source of variation	SS	df	MS	$\eta$
Attila	A	5888752	3	1962917**	73.23342
	Error	832832	6	138805.3	10.35723
	Total	8041072	11		
Boomerang	A	7364608	3	2454869**	56.62602
	Error	1400016	6	233336	10.76464
	Total	1.300577	11		

ns: no significant; \*, \*\*, \*\*\* significant at P = 5%, P = 1% and P = 0.1%

Table 3 presents the statistical significance of each variant compared to the lower fertilization rate. In the case of the Attila variety, fertilization with 60 kg N/ha was a proven statistical effect at P = 5%. On the other hand, the same fertilization rate led to a higher reliable effect for the Boomerang variety - P = 1%. For both cultivars studied, the difference in GY between N<sub>120</sub> and N<sub>180</sub> could not exceed the required

limit and remains outside the statistically significant influence. Szymańska et al. (2020) confirm that there is no significant difference between N<sub>120</sub> and N<sub>180</sub> fertilization.

Table 3. Statistical significance between fertilization variants in the formation of GY average for the test period (2015/2017)

Fertilization rates	Attila	Boomerang
	relative to the variant without fertilization	relative to the variant without fertilization
N <sub>0</sub>	-	-
N <sub>60</sub>	*	*
N <sub>120</sub>	**	**
N <sub>180</sub>	***	**

ns: no significant; \*, \*\*, \*\*\* significant at P=5%, P=1% and P=0.1%

For each variety separately, a regression analysis was performed on average for the test period. The aim was to differentiate the effect of nitrogen fertilization on GY formation. It was found that the increase in the nitrogen norm has an effect on the formation of GY. Fornari et al. (2020) come to the same conclusion and report that increasing the N application rate usually boosts crop yields. For the Attila variety, the values of the realized GY were in a positive and proven correlation

with the nitrogen rate ( $r = 0.967^{**}$ ). The mathematical model was confirmed, as well as the statistical reliability of the coefficients. GY at the four studied nitrogen rates was within the confidence interval (Figure 1).

The non-fertilizing variant and the fertilization with 180 kg N/ha achieved a lower GY than expected, with 183.1 and 173.9 kg, respectively. When applying N<sub>60</sub> and N<sub>120</sub>, higher values than the theoretical GY were reported. A higher addition to GY was found at the rate of 60 kg N/ha - 192.3 kg, compared to N<sub>120</sub> - 164.7 kg.

In the Boomerang variety, a strong and positive correlation was observed between GY and nitrogen fertilization ( $r = 0.974^{**}$ ).

The coefficients in the equations were statistically significant, and the mathematical model was reliable. Figure 2 shows that 3 out of 4 tested fertilization rates achieved lower grain yield than the theoretical prediction.

The fertilizer-free variant showed a GY of 186.3 kg less than expected. Upon fertilization with N<sub>120</sub> and N<sub>180</sub> yields were lower than the theoretical of 23.9 kg and 81.2 kg, respectively. On the other hand, the application of 60 kg N/ha resulted in more than expected 291.4 kg of grain.

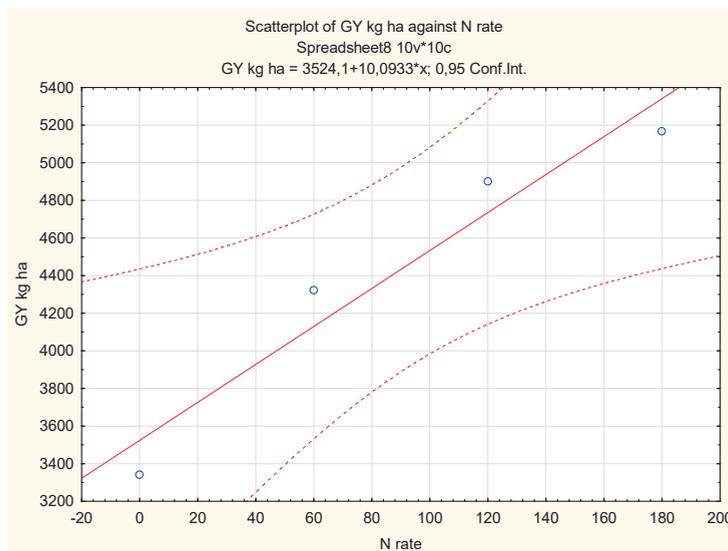


Figure 1. Theoretical change in grain yield under the influence of mineral fertilization on average for the test period (2015/2017) for the variety Attila

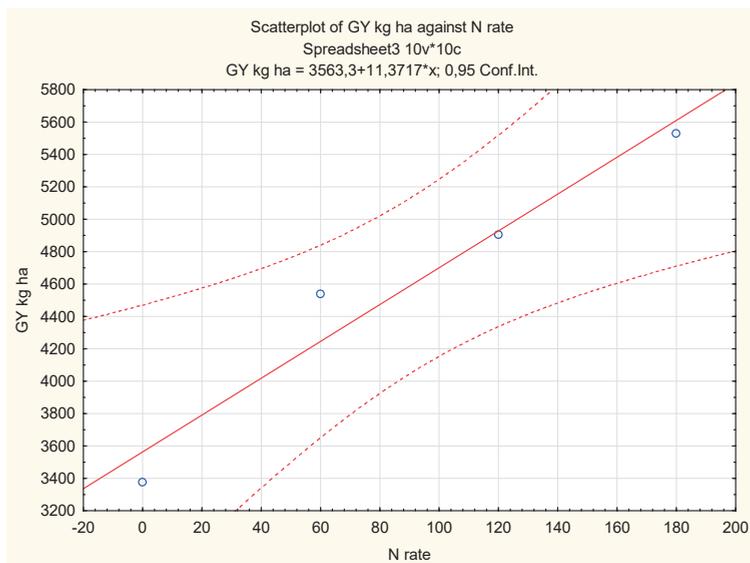


Figure 2. Theoretical change in grain yield under the influence of mineral fertilization on average for the test period (2015/2017) for the variety Boomerang

## CONCLUSIONS

In both varieties of triticale, the analysis of dispersion showed a significant effect of the tested nitrogen rates. However, when comparing the variants, the difference between  $N_{120}$  and  $N_{180}$  remained beyond statistical significance. With proven action for both triticale varieties was the difference between unfertilized control and  $N_{60}$ . The regression equations confirmed that the nitrogen rate has a strong influence on the grain yield. Both varieties showed the largest increase in grain yield compared to the theoretical prediction for fertilization with 60 kg N/ha.

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## CHERNOZEMS OF UKRAINE AND ITS EVOLUTION UNDER THE INFLUENCE OF ANTHROPOGENIC FACTORS

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### Abstract

*Materials of researches on chernozems of Ukraine, features of their genesis, distribution conditions are generalized and analyzed. The general morphogenetic characteristics of chernozems are given, which consists in a typical habitus, constitution, posture, power, unique properties and high fertility. Stability in the development of chernozems is characterized, in particular the stage of their extensive agro-technological use, which determines the intensive development of degradation processes. The role and significance of chernozems in the world of soils as a phenomenon of nature, the ideal of soil, provider and means of labor are emphasized.*

**Key words:** chernozem, fertility, degradation, plowing, soil ideal, natural phenomenon.

### INTRODUCTION

Ukraine takes a leading position in the world among the countries in which chernozems are widespread. These soils occupy 27.8 million hectares, which is 8.7% of their world area, and they are the main resource for crop production. Chernozems are the main area of agricultural land in Ukraine - 67.7%. Most crops of cereals, sugar beets, sunflowers, perennial fruit, essential ether-containing crops are grown on chernozems. Chernozems are the most developed soils and potential resources for expanding arable land in the chernozem zone are almost exhausted.

As for 2012, approximately 1.32 hectares of land, 0.92 hectares of agricultural land, 0.71 hectares of arable land and 0.59 hectares of chernozem soils were per one person in Ukraine. There is only 0.19 ha of arable land and 0.045 ha of chernozem per one person in the world (Balyuk et al., 2015).

However, high yields on chernozems are obtained only in some years, and their value is lower than in Western Europe. This is due to the irrational structure of agricultural lands, sown areas, high levels of plowing, deficient balance of biophilic elements (especially C, Ca, P, K and others) due to small doses of organic and

mineral fertilizers, imperfection of tillage technologies (extremely high availability of mechanical operations during plowing, sowing, inter-row cultivation, carrying out them outside the optimum humidity range, very low level of protection of arable lands by forest reclamation, hydraulic, chemical and agrotechnical measures and the prevalence of various degradations for this reason, significant littering of fields.

The chaotic type of land use with obvious negative (productive, environmental, social) consequences for future generations is due to inefficient level of legal protection of soil fertility, defective land cadastre, imperfection of state control over soil fertility (Havrysh, 2016).

The structures of chernozem soils of Ukraine are dominated by ordinary chernozems (10.9 million hectares), typical chernozems (6.2 million hectares) and southern chernozems (3.8 million hectares). Chernozems podzolized and degraded are 2.8 and 1.7 million hectares. In different natural and climatic zones, saline, residual-saline, shales and carbonate rocks occupy an area of about 3 million hectares.

### RESULTS AND DISCUSSIONS

Chernozems are zonal type of soil in the forest-steppe and steppe zones, stretching from the

western to eastern borders of Ukraine. Their formation took place in two soil-climatic facies: southwestern, or warm, and central, or temperate. The chernozems of the central facies are the standard and are represented by all subtypes - from podzolic to southern. The chernozems of the south-western facies are called micellar-carbonate, because carbon dioxide salts form in them thin veins located near the surface or from the surface and boil from hydrochloric acid.

The length of the chernozem strip reaches 500-550 km from north to south. Such a significant length determines the division of chernozems at the subtype level into podzolic, leached, typical, ordinary and southern. It is established by modern researches that chernozems occur in small massifs within Polissya and Maly Polissya - they are called "island" chernozems.

Chernozems of Ukraine are characterized by watershed leveled and slightly sloping surfaces of interfluvial and high floodplain terraces, which are formed mainly on forests and loess rocks, which cover mantle-like landforms. However, small massifs of chernozems are on non-loess rocks, in particular on the eluvium of carbonate, crystalline and gypsum-anhydrite rocks, are occurred.

A typical feature of chernozems is that they are confined to areas of temperate-continental climate, where evaporation often predominates over atmospheric moisture, but their predominance changes periodically.

The history of the study of chernozems of Ukraine is one of the forgotten pages of Ukrainian soil science. At the origins of the doctrine of chernozem, which preceded V.V. Dokuchaev, were Kharkiv scientists, professors N.D. Borisyak and I.F. Levakovsky. N.D. Borisyak in his work "Chernozem", published in 1852, substantiated the terrestrial-plant origin of chernozems, described their properties and methods of use. Later, V.V. Dokuchaev in his famous work "Russian Chernozem", published in 1883, described in detail not only the chernozem of Ukraine but also of Bessarabia, the Volga region, the Central Chernozem zone. The Austrian scientist L. Buber made a significant contribution to the study of the chernozems of Galicia and Podillya. In 1910 his book "Chernozems of Galicia and Podillya" was

published, which not only describes the natural conditions and properties of chernozems, but also their economic use. Began in the 20-30s of the twentieth century soil-geographical studies culminated in a consolidated map of soils of Ukraine, it is accumulated a huge amount of factual material with the characteristics of chernozems, which are summarized in G. Makhov's monograph "Soils of Ukraine" (Makhov, 1930).

Particular importance for Ukraine was the large-scale soil surveys conducted in 1957-1961, on the basis of which knowledge about the morphogenetic properties of chernozems, their use and measures to increase fertility, which is summarized in the monograph "Chernozems of the USSR (Ukraine)" in 1981.

Genetically, chernozem is a type of humus-calcium-montmorillonite soils with a complex and long history of soil formation process - from early hydromorphic to modern automorphic stages of development. Leading in the history of chernozem formation was a positive balance of biogenic substances and space energy, due to it a system of soil horizons was formed in the profile (Ao + A + Ark + NRk + Rk), enriched with humus, with significant reserves of nitrogen, phosphorus, potassium, micro elements. optimal which are forming water-air regime, active in-soil biological and biochemical processes. Huge reserves of potentially active chemical energy in humus, litter and living biomass, which reach 3-4 billion kcal per 1 ha and are the basis of high productivity of chernozems, their ability to withstand various environmental changes and ensure high productivity of plant photosynthesis.

Chernozems are characterized by different granulometric composition - from sandy to clay, inherited from the parent rock. They are characterized by a powerful humus horizon (150-180 cm), high content optimal parameters of structure density (1.0-1.2 g / cm<sup>3</sup>), porosity, moisture content, water permeability (200 mm / year), the provision of lyophilic elements (N, P, K, Ca, Mg, S) and micro elements.

Chernozems, like other soils, are a complex formation that is formed due to the interaction of biotic (soil biota) and abiotic (mineral) factors. Along with the root systems, which in

feathery virgin steppes reach a depth of 120-140 cm, it is active vertebrate and invertebrate organisms that process plant residues and cause the formation of biogenic emissions and coprogenic structure. Wellknown that 1 g of chernozem contains approximately 3.5 million individuals of living organisms, and 1 g of humus contains 55 million accordingly.

The best chernozem actually contains no more than 0.5% of above-ground substance, and no more than 10% of organic substance (humus). Everything else is a mineral mass. However, this mass thousands of years in the composition with chernozem is processed by living organisms. The combination of alive and inanimate gives reasons to call chernozem "the fourth kingdom of nature."

The value of chernozem lies in the content, reserves, quality, distribution of humus in the profile. No other soil can't be compared with chernozem by its content of humus. According to generalized results, humus reserves in the profiles of different subtypes of chernozems range from 192-247 t/ha - in the southern chernozems and 260-533 t/ha - in ordinary, to 260-560 t/ha - in typical and 200-410 t/ha - in podzolic.

Humus is a substance of complex chemical nature. The composition of chernozem humus includes black humic acids, which are especially important for soil fertility, and lighter and more soluble fulvic acids. These acids in chernozems are mainly connected with calcium, which gives them stability and high absorbency. According to the profile, the humus is distributed evenly, gradually decreasing to the bottom and reaching a depth of 60-80 cm in the southern chernozems and 150-170 cm in the typical chernozems of the Right-Bank Forest-Steppe of Ukraine.

Various processes take place in chernozems constantly - the transformation of substances, their migration, decomposition, dissolution, accumulation. The combination of these and many other processes is called the physiology of chernozem. These processes are dynamic, repeated day after day, from year to year, determining the rhythm of life of chernozem.

Chernozems have their own chemical properties. It contains all the elements of the periodic table, including silver, gold, radioactive

elements. However, it contains the most plant nutrients that are in a form accessible to them.

Chernozem has a special habit (superficies, constitution, posture), the most perfect in the world of soils: its horizons are genetically closely related, there are no sharp transitions between them. The thickness of the dark humus profile of Ukrainian chernozem reaches 220 cm in typical chernozems.

By its nature, they are generally neutral soils, but leached and podzolic chernozems have a weakly acidic reaction of the environment. This reaction of the soil solution is favorable for most cultivated plants (Kabala, 2019; Ursu et al., 2014).

The predominance of small clay parts humic acids and movable calcium in chernozem creates the preconditions for the formation of optimal water-physical and air-physical properties. Chernozems are characterized by mechanical stability and water-resistant structure, good porosity and aeration, favorable technological properties. These natural features of chernozems determine their exceptional importance in agriculture of the world and in particular of Ukraine (Pozniak, 1997).

In Ukraine, chernozems are preserved in virgin state only in the nature reserve of Mykhailivska Tsilyna (virgin land) in Sumy region, Khomutivsky steppe in Donetsk region, Streletsivsky steppe in Luhansk and Kamyany graves in Zaporizhia region, in the Kasova Hora reserve in Ivano-Frankivsk region.

Extremely high natural fertility due to the optimal combination of circulation and accumulation of carbon, nitrogen, phosphorus, calcium is the reason for long-term use of chernozems by humans. The first anthropogenic changes in the ecology and properties of chernozems were caused by a man of Stone Age who knew the fire. Motivation, and then primitive farming, which is 2-3 thousand years old, made great local changes to chernozems. Continuous development of chernozems and the introduction of industrial machinery in agriculture during the XIX-XX centuries.

The results of research indicate that the chernozems of Ukraine have undergone significant changes over the past 100 years. At the present stage of development of soil science, the provision has been established that,

depending on the culture of agriculture, agronomically valuable properties of soils may become weaker or increase. Relatively high stability in arable chernozems of morphological parameters, molecular ratio of  $\text{SiO}_2$ :  $\text{R}_2\text{O}_3$ ; the ratio of C: N, the ratio of cations in the absorption complex, indicates the stability of the parameters inherent to chernozems.

Chernozems, with few exceptions, remained untouched for millennia, as nomadic peoples dominated in the steppes. Their main task was herd cattle breeding, which provided a closed biological cycle of substances in the steppes. In the course of it, everything that was taken from chernozem returned to it. Potential soil fertility increased. This ensured the phenomenal nature of the high bioenergy of chernozem and the paradox of its stability, although human has always been very aggressive towards nature. In the seventeenth and nineteenth centuries, settled, more conscious peoples at an accelerated pace and very extensively began agricultural development of chernozems (Krupenikov, 2008).

Plowing of chernozems of the Forest-Steppe of Ukraine reaches 85%, and in the steppe it is even bigger - more than 91%. But in recent years these indexes have declined, however they still remain high. Currently, plowing of soils in Ukraine is 54%. However, the plowing of chernozems during a long time almost without the use of fertilizers has led to significant changes in the structure, composition and properties of soils. Plowing and the long process of agricultural use have changed the structure, composition and properties of soils, thereby disrupting the normal flow of energy, reducing the level of humus recovery and the release of biophilic elements connected in plant and animal biomass and soil humus. There was a loss of structure and self-compaction of soil mass. Heavy agricultural instruments intensify this process, especially on wet soils. Chernozems lose chemically bound energy in humus, aggregation and porosity, which are important for saving fertility. Over the last 40 years, so much energy has been taken from chernozems that it would be enough to boil the Black Sea (Rudenko, 2008).

Plowing, development, long-term cultivation of chernozems lead to a significant reduction

(obviously not less than 2-6 times) in the number of different organisms and chernozems start to "sterilize".

As a result of chernozem acquires a clearly different physical condition and its corresponding parameters. If the compaction of root-containing horizons of chernozems increases in terms of structure density from the optimum of  $1.1-1.2 \text{ g/cm}^3$ , which is typical for chernozems to  $1.4-1.6 \text{ g/cm}^3$ , it has a negative effect on their fertility. Compaction of chernozems causes a decrease in water permeability, porosity decreases by 10-20%, the moisture of wilting of plants increases, it becomes more difficult to cultivate the soil. Drought and waterlogging have a stronger effect on compacted chernozems, causing plant oppression. Yields on compacted chernozems are reduced 15-30%, and during the consolidation to  $1.5-1.6 \text{ g/cm}^3$  it lose 50-60%.

Plowing of chernozems promotes the development of erosion processes. The predominant types of erosion in the chernozem zone of Ukraine are water and wind erosion. Eroded arable land is 30% of arable. Eroded chernozems - 25%. Among them, weakly eroded - 18%, medium eroded - 5% and strongly eroded - 2%. Erosion is a kind of guillotine of chernozems, which causes loss of habitus, reduction of humus content, deterioration of properties and generally reduction of chernozems fertility. The structure of the soil deteriorates intensively, appearing itself in the growth of boulders. On the territory of Ukraine for 130 years after V.V. Dokuchaev's research the average annual losses of humus in the arable horizon of chernozems are from 21 to 40%, or 0.5-0.9 t/ha. (Russian chernozem, 1983). For example, the content of humus in the upper 30-centimeter horizon of chernozem typical in the Mykhailivska Tsilyna is 9.5-10%, and in the same chernozems that are in agricultural use - 4.5-5%.

Long-term researches of chernozems of Ukraine have shown that the average annual losses of humus in typical chernozems are 0.7-0.9 t/ha, ordinary - 0.5-0.7 t/ha, southern - 0.3-0.6 t/ha. As a result of the dehumidification process, as well as under the influence of excessively intensive tillage with energy-intensive heavy machinery, unbalanced use of mineral fertilizers

(mostly physiologically acidic), the physical properties of chernozems deteriorate.

Extensive, irrational use, non-observance of crop rotations, reduction of perennial grasses, insufficient application of organic fertilizers, etc. significantly affect the intensity of chernozem degradation. In recent decades, the amount of absorbed calcium in typical chernozems has decreased to 26-37%, water-resistant aggregates (over 0.25 mm in size) to 33%, mineral nitrogen - to 34-40%, soluble phosphates - to 39-40%, exchangeable potassium - to 22-24% (Nosko, 2006).

In recent years, with the introduction of market-oriented sorts of wheat, corn, sunflower, rapeseed while using of modern cultivation technologies in many agricultural holdings get high yields on the large areas. However, in such fields there is an acute deficit of nutrients, especially nitrogen, and all nutrients are wasted from chernozem reserves. In this way, the laws of agriculture are violated, on the basis of which it can be stated that such management of chernozems will further reduce fertility, as chernozems can not withstand the increased capacity and predatory use (Kharytonov et al., 2004).

One of the powerful factors of human intervention in the soil-ecological system of the steppes is the irrigation of chernozems in Ukraine. If plowing and fertilizing affect mainly the upper soil horizons, then irrigation covers a much larger layer of soil-subsoil. That is why the effects of irrigation are too strong. Water, salt, heat, microbiological, gas and nutrient regimes change under the influence of irrigation. There are new processes not yet typical for chernozems - raising the groundwater level, flooding, secondary salinization, salinization, alkalization, removal of nutrients, including calcium, deterioration of physical condition, crust formation on the surface, cracking and more. In recent decades, the area of irrigated lands in Ukraine has decreased significantly and is actually 600-700 thousand hectares (Balyuk et al., 2015).

Significant damage to chernozems is caused by local waterlogging, salinization, pollution and clogging with production and consumption

wastes, pollution by radionuclides and heavy metals. Irreversible damage is caused by the destruction of chernozems in the course of open-cast mining and the construction of roads, industrial and other facilities.

In recent years, an extremely important problem has arisen - the military degradation of chernozems in the Donbass. Highly productive soils are being destroyed on the territory of hostilities, chernozems have suffered and are experiencing irreparable military degradation, which leads to disruption of morphological structure of the profile, mixing of genetic horizons, changes in composition and properties, appearance of unusual inclusions in the soil - foreign bodies, shell fragments, shell fragments, intensive compaction by heavy military equipment, violation of the ground cover due to the rupture of mines, grenades, construction of trenches, dugouts, dugouts, trenches, etc., the formation of large areas of funnels, ditches, pits, violating the structures of the soil rush, its homogeneity and integrity. It is necessary to resuscitate and rehabilitate such soils in order to improve their condition. Solving of this problem will be a very important stage in the revival of Donbass.

The degradation of chernozems in Ukraine has not yet turned into a catastrophe (except for the territory of hostilities in the Donbass). It is easy to predict it, but very difficult to overcome.

Chernozem is an ideal, a standard of perfection in the world of soils in many countries around the world, including Austria and Germany. In these countries, 2005 was declared the Year of Chernozem and on this occasion in a postage stamp with the image of chernozem with a thickness of 120-130 cm was released (Altermann et al., 2005). To achieve food independence, productive soils are artificially created, taking as an example natural chernozems. In a broad sense, the welfare of Ukraine is largely based on the country's natural resources, including - chernozem - perennial breadwinner. It is no coincidence that a sample of chernozem from the Dobrovlychivsky district of the Kirovohrad region is in the Laboratory of Land Resources of Europe as a "standard of chernozem".

## CONSLUSIONS

Based on the role and importance of chernozem in nature and public life, on its aesthetic value, humanity highly appreciates this natural phenomenon. Scientific works, monographs, works of art are dedicated to him, also monuments have been built, and in some museums and educational institutions of the world there are collections of monoliths of virgin soils (Pozniak et al., 2019).

From an aesthetic point of view, chernozem is just a beautiful soil, it has an incomparable color in the virgin state, shimmers and sparkles on the edges of structural units (on the ridges of the furrow during plowing), resembling the color of a crow's wing. And even Mansell's color chart is not able to identify and name this color. In the spring plowing chernozem rises in a mirage, then falls, trembles in the spring anxiety, breathes, and all-around smells of chernozem. All these forms the aesthetic grandeur of chernozem and its ability to revive.

Being a breadwinner and a means of labor, ecological perfection and aesthetic value, chernozem is a strong and at the same time defenseless. Strength and defenselessness - such a unity of opposites is contained in chernozem.

Important conditions for increasing the productivity of chernozems, their efficient use and protection include minimizing the size of alienation of chernozems for non-agricultural use, introduction of a set of measures to prevent degradation, construction of the most advanced irrigation systems, use of irrigation water that fits the quality standards. the use of agricultural machinery, which eliminates the negative impact on the physical properties of soils, control and prevention of pollution by industrial waste and pesticides, to achieve agricultural culture in combination with a set of chemicals and land reclamation, reproduction of chernozems affected by human activities and more.

To overcome the spread of chernozem degradation, it is necessary to develop and implement legislation on soil protection, organization and realization of soil monitoring, transition to landscape-ecological land use and soil-protective agriculture.

Chernozem is a world heritage, so preserving and increasing its unique properties is an important task and responsibility for all humanity.

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# CROP SCIENCES



## ROLE OF FOLIAR SPRAY OF SALICYLIC ACID AND SPERMINE ON SOME CHARACTERISTICS OF ISABGOL (*Plantago ovata* Forssk)

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### Abstract

In order to investigate the effect of drought stress on some physiological and biochemical characteristics of isabgol, a study was carried out as a split-plot factorial experiment based on a randomized complete block design with 18 treatments and three replications, at Iran, during growing seasons of 2016 and 2017. Irrigation treatment included: normal irrigation, cutoff irrigation at the flowering stage and cutoff irrigation at seed filling stage which considered as main-plots. Salicylic acid with three levels (0, 0.4 and 0.8 mM) and Spermine with two levels (0 and 0.02 mM) was assigned in sub-plots. The results of experiment showed that the highest grain yield under irrigation conditions was obtained by foliar spraying of 0.8 mM Salicylic acid and 0.02 mM Spermine at seed filling stage. Application of Salicylic acid at concentration of 0.8 mM along with or without Spermine improved the amount of the cell membrane stability index under different irrigation levels. The highest catalase activity in both years of the study was attributed to normal irrigation treatment with the foliar application of Salicylic acid at 0.4 mM concentration and with or without Spermine. Application of SA<sub>0.8</sub>mM and Spm<sub>0.02</sub> mM under cutoff irrigation condition increased the most of the measured features compared to control, which indicates the positive effect of these compounds.

**Key words:** antioxidant defense system, drought stress, electrolyte leakage, herbal plant, osmotic compounds.

### INTRODUCTION

Isabgol (*Plantago ovata* forssk) of the family *Plantaginaceae* is an important medicinal plant, which is widely used in the textile, military, food, cosmetics and pharmaceutical industries (Mardani Karani, 2013). Drought stress is an important environmental factor affecting crop productivity worldwide. Drought stress affects mainly through disturbance of the balance between the productions of reactive oxygen species (ROS) and antioxidant defense mechanism and cause oxidative stress (Nasibi, 2011). Under such condition, the high activity of antioxidant enzymes and high levels of non-enzyme antioxidants are very important for plant tolerance to stress (Nasibi, 2011). Cell membrane is the first site of damage under stress conditions. By reducing the potential of cellular water, the accumulation of compatible osmolytes involved in osmoregulation like proline and soluble sugars allows additional water to be taken up from the environment and for a short time; the balance of water is maintained within plant cells. Thus, osmotic adjustment is an adaptation mechanism to

increase drought tolerance (Inze and Montagu, 2000; Kumar et al., 2003). An alternative approach is to apply exogenous phytohormones (polyamines, salicylic acid and gibberellic acid), plant growth promoting rhizobacteria or other effective compounds that can protect plants under limited moisture (Hara et al., 2012). Salicylic acid (SA) and Spermine (Spm) are important phytohormone and are involved in responding to biotic stresses. Moradi and Pourghasemian (2018) reported that contents of carotenoid, soluble sugars, proline and phenol in Marigold were significantly increased by decreasing amount of irrigation water, while foliar application of salicylic acid with 1.5 mM concentration; increased the plant dry weight, carotenoid contents and soluble sugars relative to the control conditions. In a study on exogenous application of salicylic acid under water stress conditions, the amount of secondary metabolites, chlorophyll, anthocyanin, protein, phenol and flavonoid in the *Melissa officinalis* L. increased (Jamal Omidi et al., 2018). In another study on the Centipedegrass Mutant, the increased activity of antioxidant enzymes such as catalase (CAT), ascorbate

peroxidase (APX), glutathione reductase (GR), and superoxide dismutase (SOD) was reported in after external application of putrescine, spermidine and spermine (Liu et al., 2017). Combined application of 500  $\mu\text{M}$  salicylic acid and 1  $\mu\text{M}$  spermine increased seed protein content, oil content and proline concentration of castor bean leaves under drought stress conditions (Tadayon and Izadi, 2015). The aim of current study was (a) to investigate the effect of Salicylic acid and Spermine application on seed yield and (b) to understand physiological mechanism/s involved under water stress condition.

## MATERIALS AND METHODS

### Experiment

To evaluate the response of some physiological and biochemical characteristics of isabgol response to water deficit and application of salicylic acid and spermine, a two-year experiment (2016 and 2017) was conducted in the research field of Gonbad Kavous University, located in Golestan province, Iran in 55°21'E, 37°26'N, 45 m above sea level with 450 mm mean 10 years precipitation. The meteorological information during the experiments are shown in Table 1.

The land soil texture was silt-loam, with bulk density of 1.5  $\text{g}/\text{cm}^3$ , pH of 7.92, electrical

conductance of 1.2  $\text{dS}\cdot\text{m}^{-1}$ , field slope of  $\leq 0.2$ , organic carbon of 1.11%, total N of 0.11%, available P of 21.2  $\text{mg}\cdot\text{kg}^{-1}$  and K of 504  $\text{mg}\cdot\text{kg}^{-1}$ . The experiment was arranged as a split plot factorial based on randomized complete block design with 18 treatments and three replications. Treatments include: three irrigation levels (Control (non-stress), irrigation cutoff at flowering stage (severe stress) and irrigation cutoff at seed filling stage (moderate stress)), three Salicylic acid level ( $\text{SA}_0$  = sprayed with distilled water,  $\text{SA}_{0.4}$  = sprayed 0.4 mM of Salicylic acid,  $\text{SA}_{0.8}$  = sprayed 0.8 mM of Salicylic acid) and two Spermine levels ( $\text{Spm}_0$  = sprayed with distilled water,  $\text{Spm}_{0.02}$  = sprayed 0.02 mM of Spermine). Irrigation was used as main-plot, Salicylic acid application and Spermine spraying was as sub-plot. Isabgol (with 98% viability and seed purity) was hand sown in 0.5-1 cm soil depth on 7 March 2016 and 2017. In this experiment nitrogen and phosphorus fertilizers were added respectively with a dose of 75  $\text{kg}\cdot\text{ha}^{-1}$  urea and 10  $\text{kg}\cdot\text{ha}^{-1}$  triple super phosphate, based on soil test and fertilizer recommendations for isabgol. The exogenous Salicylic acid (molecular weight 138.1, Sigma) and Spermine (molecular weight 202.3, Sigma) were applied during plant budding (flowering stem production), flowering and seed filling stages.

Table 1. Metrological statistics of Gonbad Kavous in 2016 and 2017

	Precipitation (mm)		Average temperature ( $^{\circ}\text{C}$ )		Relative humidity (%)	
	2016	2017	2016	2017	2016	2017
March	23	35.6	9.3	12.8	79	75.2
April	65.1	37.2	15.2	14.8	77	76
May	27.8	30.4	22.1	21.4	72	70
Jun	3.2	0.3	23.3	25.2	72	59

Soil moisture content at field capacity and permanent wilting point were 0.9 and 0.7% (equivalent to a weigh moisture of 16.8 to 21.6), respectively (Walter and Gardener, 1986).

The depth of irrigation was determined based on the average soil water content that was calculated by following equation (Allen et al., 1998):

$$dw = \frac{(\theta_{m1} - \theta_{m2})}{100} \times \rho_b \times ds \quad (1)$$

In this equation;  $dw$  (cm) represents depth of irrigation,  $\theta_{m1}$  represents initial weight moisture (FC) (%),  $\theta_{m2}$  represents secondary weighs moisture (WP) (%),  $\rho_b$  represents bulk density ( $\text{g}/\text{cm}^3$ ) and  $ds$  represents depth of soil (cm).

Irrigation (with furrow irrigation system) was carried out on all plots until the complete plant establishment (four-leaf stage) as needed. Then, soil moisture content was maintained before the application of stress treatments for all experimental plots.

## Measurements

After biological maturity, 10 plants were randomly sampled from each plot to measure membrane stability and electrolyte leakage.

We harvested two square meters of three central rows from each plot to determine the seed yield.

Sairam et al. (1994) method was followed for analysis of membrane stability index (MSI).

$$MSI = [1 - (EC1/EC2)] \times 100 \quad (2)$$

Electrolyte leakage (EL) percentage was calculated by the following equation as proposed by Tas and Basar (2009).

$$EL = EC1/EC2 \times 100 \quad (3)$$

## Statistical analysis

The data were subjected to analysis of variance (ANOVA) using the SAS package (version 9.1).

The LSD test was applied to test significance of treatment means at 0.05 and 0.01 levels of probability. In order to investigate the uniformity of variances between two year Levene test was used.

## RESULTS AND DISCUSSIONS

Analysis of variance (ANOVA) (Table 2) of the first year of the experiment revealed that the two-way interaction effects of irrigation and Salicylic acid were statistically significant on all characteristics ( $P \leq 0.01$  and  $P \leq 0.05$ ). The two-way interaction irrigation and Spermine had a significant effect on some characters for example seed yield and catalase activity. Salicylic acid and Spermine had a significant effect on seed yield and catalase activity. Analysis of variance (ANOVA) revealed that the three-way interaction effects of irrigation and Salicylic acid and Spermine were statistically significant on all characteristics ( $P \leq 0.01$  and  $P \leq 0.05$ ). Second year results (Table 3) revealed that the two-way interaction effects of irrigation and Salicylic acid plus the three-way interaction effects of irrigation and Salicylic acid and Spermine were statistically significant on all characteristics ( $P \leq 0.01$  and  $P \leq 0.05$ ). The two-way interaction irrigation and Spermine had a significant effect on measured characteristics except for membrane stability index and electrolyte leakage.

Table 2. Variance analysis effect of cutoff irrigation, salicylic acid and spermine foliar application on some morphological and physiological traits of isabgol in 2016

Source of variation	Rep.	irrigation (IR)	Error (Ea)	Salicylic acid (SA)	Spermine (Spm)	IR×SA	IR×Spm	SA×Spm	IR×SA×Spm	Error (Ebc)	C.V (%)
Df	2	2	4	2	1	4	2	2	4	30	-
Seed yield	*	**	960.5	**	**	**	**	**	**	854	7.25
Membrane stability index	Ns	**	0.56	**	Ns	**	Ns	Ns	*	0.65	0.86
Electrolyte leakage	Ns	**	0.56	**	Ns	**	Ns	Ns	*	0.65	12.65
Catalase activity	Ns	**	0.44	**	**	**	**	**	*	0.12	4.38

Ns, \* and \*\* are Non-Significance and Significance at  $P \leq 0.05$  and  $P \leq 0.01$ , respectively

Table 3. Variance analysis effect of cutoff irrigation, salicylic acid and spermine foliar application on some morphological and physiological traits of isabgol in 2017

Source of variation	Rep.	irrigation (IR)	Error (Ea)	Salicylic acid (SA)	Spermine (Spm)	IR×SA	IR×Spm	SA×Spm	IR×SA×Spm	Error (Ebc)	C.V (%)
Df	2	2	4	2	1	4	2	2	4	30	-
Seed yield	*	**	960.5	Ns	**	**	*	**	**	364	4.22
Membrane stability index	Ns	**	6.00	**	Ns	**	Ns	**	**	10.15	3.80
Electrolyte leakage	Ns	**	6.00	**	Ns	**	Ns	**	**	10.15	19.70
Catalase activity	Ns	**	0.93	*	**	**	**	**	**	0.44	6.37

Ns, \* and \*\* are Non-Significance and Significance at  $P \leq 0.05$  and  $P \leq 0.01$ , respectively

### Membrane stability index (MSI)

The interactions between irrigation levels and spray treatments in the first year of the experiment showed that SA<sub>0.08</sub> mM+Spm<sub>0</sub> mM application increased membrane cell stability in plants under normal irrigation and cutoff irrigation at seed filling stage. The highest amount of MSI was obtained in SA<sub>0.8</sub> mM+Spm<sub>0</sub> mM with 97.63%. There was no significant difference in MSI between SA and Spm spraying under cutoff irrigation condition at the flowering stage (Table 4). The results of

the comparison of the mean of second-year data also indicated that the cell membrane stability of some of the treatments was more than control under different levels of irrigation and foliar application of Salicylic acid and Spermine (Table 4). The highest and lowest cell membrane stability under normal irrigation and non-foliar application under severe stress conditions were assigned to foliar spray SA<sub>0.8</sub> mM+Spm<sub>0</sub> mM with 94.68 and 71.06 percent, respectively (Table 4).

Table 4. Interaction of irrigation × Salicylic acid × Spermine in the first and second year of experiment, respectively, on membrane stability index percentage of isabgol

Treatments	Control (non-stress)						Irrigation cutoff at flowering stage						Irrigation cutoff at seed filling stage					
	SA <sub>0</sub>		SA <sub>0.4</sub>		SA <sub>0.8</sub>		SA <sub>0</sub>		SA <sub>0.4</sub>		SA <sub>0.8</sub>		SA <sub>0</sub>		SA <sub>0.4</sub>		SA <sub>0.8</sub>	
Year	Spm <sub>0</sub>	Spm <sub>0.02</sub>	Spm <sub>0</sub>	Spm <sub>0.02</sub>	Spm <sub>0</sub>	Spm <sub>0.02</sub>	Spm <sub>0</sub>	Spm <sub>0.02</sub>	Spm <sub>0</sub>	Spm <sub>0.02</sub>	Spm <sub>0</sub>	Spm <sub>0.02</sub>	Spm <sub>0</sub>	Spm <sub>0.02</sub>	Spm <sub>0</sub>	Spm <sub>0.02</sub>	Spm <sub>0</sub>	Spm <sub>0.02</sub>
2015-2016	92.97 fg	92.08 gh	93.85 def	94.82 cd	96.57 ab	95.40 bc	92.04 gh	91.44 h	91.42 h	91.44 h	91.89 gh	92.78 fg	92.62 fgh	93.51 def	94.71 cde	93.46 cf	97.63 a	96.56 ab
2016-2017	80.97 f-i	86.73 b-e	88.86 bc	81.83 e-h	94.68 a	90.80 ab	71.06 j	78.02 hi	75.86 ij	83.48 d-g	84.61 e-g	76.45 i	88.11 bcd	89.18 bc	79.89 ghi	83.48 d-g	88.90 bc	85.99 b-e

Means with similar letters did not show statistically significant differences at 5% level of probability according to LSD  
 SA<sub>0</sub>= No salicylic acid (water spray), SA<sub>0.4</sub>= Spraying 0.4 mM of salicylic acid, SA<sub>0.8</sub>= Spraying 0.8 mM of salicylic acid. Spm<sub>0</sub>= not using spermine (water spray), Spm<sub>0.02</sub>= Spraying 0.02 Mm of spermine.

### Seed yield

We can infer that Salicylic acid and Spermine spraying could increase the grain yield of isabgol by stimulating the physiological processes that cause an active transfer of photosynthetic products from source to sink. In a study, Afsharmanesh et al. (2008) stated that the yield of isabgol under severe stress conditions (irrigation after 25% field capacity) was 43% lower than that of moderate stress (irrigation after 75% field capacity). Rezaichianah and Pirzad (2014) reported a 13% increase in black cumin grain yield under water stress condition with 0.5 mM salicylic acid application. The results of this study are also consistent with the results of Ramroudi et al. (2011).

### Membrane stability index (MSI)

Water stress causes a disruption between the production of reactive oxygen species and antioxidant defense, which will cause oxidative

stress. As soon water availability of plants reduces then stomata would be closed and thus the flow of carbon dioxide reduces. Reducing carbon dioxide does not only directly reduce the activity of rubisco carboxylase in the Calvin cycle; it also increases the production of reactive oxygen species by incomplete oxygen recovery (Farooq et al., 2009). In a study Naghashzadeh (2014); MSI, as affected by different irrigation regimes, was decreased by increasing drought stress. He reported that well-watered had the highest MSI of all irrigation regimes and severe drought stress was 28% lower than well-watered conditions. A similar result was reported that exogenous Salicylic acid and Spermine was effective in enhancing the cell membrane stability under water stress. Bandurska and Stroinski (2005) reported that plant treatment with SA before drought stress reduced a damaging action of water deficit on the cell membrane in leaves. The increase of cell membrane stability with

300 ppm salicylic acid under drought stress conditions was reported by Sibi et al. (2012). Application of spermine and putrescine increased drought tolerance through reducing the electrolyte leakage, increasing compatibility osmolytes and antioxidant enzyme activity (Amraee et al., 2016).

### Electrolyte leakage (EL)

The positive effect of the use of polyamines on the reduction of ion cell membrane leakage has also been reported in other studies (Kubis et al., 2014). Masoumi et al. (2010) stated that drought stress causes a significant decrease RWC in the *Kochia Scoparia* leaves and increase electrolyte leakage compare with control.

### CONCLUSIONS

Application of salicylic acid in case of moderate and severe water stress can increase seed yield, membrane stability index. Application of salicylic acid at appropriate concentrations can alleviate adverse effects of water deficit stress on growth and performance of the isabgol plants when applied at a proper time which can be determined taking into account the climatic conditions of the production area.

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## IMPACT OF FOLIAR TREATMENT WITH HUMATE FERTILIZER ON THE BIOPRODUCTIVE INDICATORS OF A NATURAL MEADOW OF *Chrysopogon gryllus* TYPE IN CONDITIONS OF THE CENTRAL BALKAN MOUNTAIN

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### Abstract

The experiment was conducted in the region of the Central Balkan Mountain (Bulgaria) in the period 2013-2015. Humate fertilizers were the objective for the present research was humate fertilizers (Phosphorus humate, Boron humate and Molybdenum humate) applied individually and in combination (at the stage of active vegetation of grass species) on the natural grass stand of *Chrysopogon gryllus* type. Grass stands treated with Molybdenum humate alone (160 ml/da) formed the highest yield of fresh (570.0 kg/da) and dry (195.6 kg/da) mass, which was proven ( $P < 0.01$ ). While the variants with foliar treatment by a combination of biofertilizers, such as Phosphorus humate (250 ml/da) + Boron humate (100 ml/da) + Molybdenum humate (100 ml/da), registered the lowest values (470.0 kg/da of fresh mass and 155.7 kg/da of dry mass). The percentage and species share of the main biological groups (grasses, legumes, motley grasses) in the natural grass stand changed in a positive way from the first to the third experimental year. The share of both main species *Chrysopogon gryllus* (characterizing the grass community) and motley grasses decreased, while there was an increase of *Agrostis capillaris* and legume meadow grasses (*Trifolium campestre* and *Lotus corniculatus*) in the above ground mass. The above data presupposes the formation of biomass with better qualitative and quantitative indicators.

**Key words:** *Chrysopogon gryllus* L., natural grass stand, productivity, botanical composition.

### INTRODUCTION

The treatment of agricultural crops with biofertilizers is one of the main emphasis in the program for building sustainable agriculture and maintaining soil fertility (Kostadinova & Popov, 2012; Saini & Kumar, 2014; Georgiev et al., 2017; Bozhanska et al., 2019; Guilherme et al., 2020). The application of organic products of natural origin has a beneficial effect on plants, development and nutritional regime of plants (Churkova, 2012; Churkova & Bozhanska, 2016). Organic products with complex action and high content of labile humate substances perform a corrective function in the integrated "soil-plant" system. Biofertilizers contain formulations of live microorganisms, enzymes and microelements in an easily accessible form that facilitate seed germination and viability (Han et al., 2007; Bhardwaj et al., 2014; Bozhanska & Naydenova, 2020). The introduction of humate products stimulates the growth of the root

system, increases the utilization rate of nutrients (Datta et al., 2011; Churkova, 2013a,b) and improves the photosynthetic activity and carbohydrate metabolism (Sengalevich, 2007) of plants. The realized aboveground mass is of better quality and is used more efficiently as a source of animal feed (Naydenova et al., 2013; Naydenova et al., 2014; Bozhanska et al., 2017; Churkova, 2019). Methodical and regulated fertilization creates conditions for changes in the phytocenological and qualitative profile of natural grass communities (Samuil et al., 2013; Iliev, 2018) by stimulating or suppressing the share of main grass species in meadow and pasture grass stands (Marușca et al., 2014). As a result, there is degradation in the percentage share of low-quality plant species and an advantage of grasses and legumes, which provide easily digestible, energy and protein balanced feed biomass (Naydenova et al., 2013; Vasileva & Enchev, 2018).

In Bulgaria, the researches related to the application of humate fertilizers on natural grass stands are limited and insufficient. Therefore, the objective of the present study is to observe the impact of annual foliar treatment with humate fertilizer on the bioproductive indicators of a natural meadow of *Chrysopogon gryllus* type in conditions of the Central Balkan Mountain.

## MATERIALS AND METHODS

The experiment was conducted in the spring (20<sup>th</sup> of April) of 2013 on a natural grass stand of *Chrysopogon gryllus* type (the predominant species were bunch grasses and common bent) in the main part of the Central Balkan Mountain. The experimental design was a block method with 4 replications. The plot size was 5 m<sup>2</sup>.

In terms of climate, the territory belongs to the Pre-Balkan (Mountain) climate region of the temperate-continental climate subregion (Sabev & Stanev, 1963). Temperatures bear the marks of continental influence. The average annual temperature is 10-11°C and is characterized by

territorial differentiation (from north to south) with increasing altitude (Ninov, 1997). The distribution of precipitation is uneven (maximum in summer: 309.0 mm and minimum in winter: 168.0 mm). In the spring and autumn the amount of precipitation is 242.0 mm and 209.0 mm, respectively, and the annual amount of precipitation reaches from 567.0 mm to 1200.0 mm.

The manifestation of climatic indicators is an important factor (Andreeva et al., 2015) determining the impact of humate fertilizers included in the experiment on the development and productivity of natural grass stand. The vegetation precipitation in 2013 was 658.7 mm at an average air temperature of 15.4°C (Figure 1). The lowest average air temperature (14.8°C) during the vegetation period, was registered in the second experimental year (2014), when the amount of precipitation reached maximum values (1045.9 mm) compared to the other experimental years. In 2015, the sum of precipitation for the period March-October was 787.9 mm, and the average air temperature was 15.9°C.

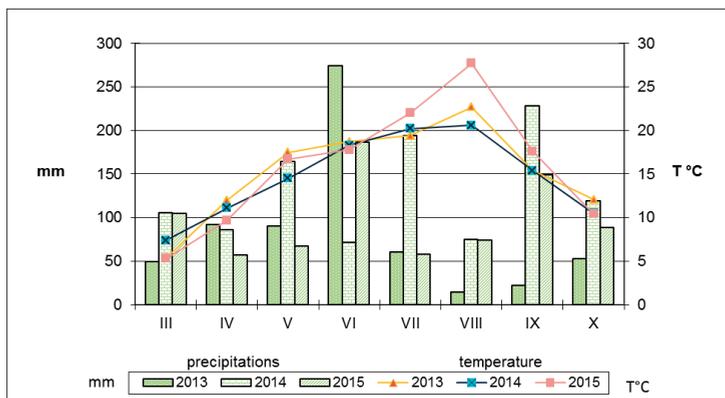


Figure 1. Average monthly temperatures (°C) and monthly precipitation amounts (mm) for the vegetation period (March-October) 2013-2015

### Variants in the study were

1. Control (C)
2. Phosphorus humate (300 ml/da) - PH (300 ml/da)
3. Boron humate (160 ml/da) - BH (160 ml/da)
4. Molybdenum humate (160 ml/da) - MH (160 ml/da)
5. Phosphorus humate (250 ml/da) + Boron humate (100 ml/da) + Molybdenum humate

(100 ml/da) - PH (250 ml/da) + BH (100 ml/da) + MH (100 ml/da).

### Characteristics of the applied high polymeric humate fertilizers

**Phosphorus humate contains:** N - 6%; P - 18%; Organic carbon - 0.4%; Active substance - 98%; Organic substances; Humate acids;

Fulvic acids; Amino acids; Valine; Glutamine; Methionine; Lysine; Vitamins and Microelements.

**Boron humate contains:** N - 6%; B - 5%; Organic carbon - 0.4%; Active substance - 98%; Organic substances; Humate acids; Fulvic acids; Amino acids; Valine; Glutamine; Lysine; Methionine; Vitamins and Microelements.

**Molybdenum humate contains:** N - 6%; B - 5%; Organic carbon - 0.4%; Active substance - 98%; Organic substances; Humate acids; Fulvic acids; Amino acids; Valine; Glutamine; Methionine; Lysine; Vitamins and Microelements.

The foliar treatment with the tested fertilizers was applied once, every year. The working solution was imported with a back sprayer during the period of active vegetation for the grass species. During the three-year study period, three mowings were carried out on the grass cover (one in each of the experimental

years), in the phase of hay hay harvesting stage of the grass stand, i.e. when the grasses are in the tasseling/ear formation and the legumes in the beginning of flowering.

Experimental data were statistically processed by analysis of variance (ANOVA).

## RESULTS AND DISCUSSIONS

On average for the period, the highest yield of fresh (570.0 kg/da) and dry (195.6 kg/da) mass was registered in the variants with foliar treatment with Molybdenum humate (160 ml/da) - Tables 1 and 2. The amount of fresh mass of that variant exceeded the control by 33.1% (P < 0.01). The grass stands treated with the combination of Phosphorus humate (250 ml/da) + Boron humate (100 ml/da) + Molybdenum humate (100 ml/da), registered the lowest values (470.0 kg/da of fresh mass and 155.7 kg/da of dry mass).

Table 1. Fresh mass yield (kg/da) of natural meadow of *Chrysopogon gryllus* type over the years and on average for the period of 2013-2015

Variants	2013		2014		2015		2013-2015	
	kg/da	% to C	kg/da	% to C	kg/da	% to C	kg/da	% to C
Control (C)	440.0	100.0	440.0	100.0	405.0	100.0	428.3	100.0
FH (300 ml/da)	500.0	113.6	540.0	122.7	450.0	111.1	496.7	116.0
BH (160 ml/da)	520.0	118.2	420.0	95.5	640.0	158.0	526.7	123.0
MH (160 ml/da)	550.0	125.0	460.0	104.6	<b>700.0</b>	<b>172.8*</b>	<b>570.0</b>	<b>133.1*</b>
FH (250 ml/da) + BH (100 ml/da) + MH (100 ml/da)	490.0	111.4	450.0	102.3	470.0	116.1	470.0	109.7
<b>LSD<sub>0.05</sub></b>	<b>144.9</b>	<b>32.9</b>	<b>103.8</b>	<b>23.6</b>	<b>273.7</b>	<b>67.6</b>	<b>125.6</b>	<b>29.6</b>
<b>LSD<sub>0.01</sub></b>	<b>203.4</b>	<b>46.2</b>	<b>145.7</b>	<b>33.1</b>	<b>384.2</b>	<b>94.9</b>	<b>176.3</b>	<b>41.5</b>
<b>LSD<sub>0.001</sub></b>	<b>287.2</b>	<b>65.3</b>	<b>205.7</b>	<b>46.7</b>	<b>542.4</b>	<b>133.9</b>	<b>248.9</b>	<b>58.6</b>

Table 2. Dry mass yield (kg/da) of natural meadow of *Chrysopogon gryllus* type over the years and on average for the period of 2013-2015

Variants	2013		2014		2015		2013-2015	
	kg/da	% to C	kg/da	% to C	kg/da	% to C	kg/da	% to C
Control (C)	150.4	100.0	147.4	100.0	191.4	100.0	163.1	100.0
FH (300 ml/da)	158.9	105.7	172.4	116.9	147.4	77.0	159.6	97.9
BH (160 ml/da)	167.0	111.1	144.0	97.7	227.5	118.8	179.5	110.1
MH (160 ml/da)	183.7	122.2	153.1	103.9	250.0	130.6	195.6	119.9
FH (250 ml/da) + BH (100 ml/da) + MH (100 ml/da)	155.4	103.4	145.9	98.9	165.8	86.6	155.7	95.5
<b>LSD<sub>0.05</sub></b>	<b>47.1</b>	<b>31.4</b>	<b>34.2</b>	<b>23.3</b>	<b>97.9</b>	<b>51.1</b>	<b>43.4</b>	<b>26.6</b>
<b>LSD<sub>0.01</sub></b>	<b>66.2</b>	<b>44.1</b>	<b>48.1</b>	<b>32.7</b>	<b>137.5</b>	<b>71.8</b>	<b>61.0</b>	<b>37.4</b>
<b>LSD<sub>0.001</sub></b>	<b>93.4</b>	<b>62.3</b>	<b>67.9</b>	<b>46.2</b>	<b>194.1</b>	<b>101.3</b>	<b>86.0</b>	<b>52.8</b>

In the first experimental year, the yield of **fresh mass** from a natural meadow of *Chrysopogon gryllus* type varied from 440.0 kg/da (Control)

to 550.0 kg/da (MX - 160 ml/da). In percentage terms, the excess of the indicator in the treated grass stands compared to the control was from

11.4 to 25.0%. In the year (2014) with the lowest air temperature and the highest amount of vegetation precipitation, fertilization with Phosphorus humate (300 ml/da) had the highest effect on the productivity of the natural grass stand. The values of the indicator exceeded the control by 22.7%. In 2015, the effect of foliar treatment with Molybdenum humate (160 ml/da) led to a proven excess in fresh mass yield by 72.84% ( $P < 0.05$ ).

In the years of the experimental period, the application of the studied humate fertilizers did not significantly affect the yield of **dry mass** from a natural meadow of *Chrysopogon gryllus* type. In the first and third experimental years, the variants treated with Molybdenum humate (160 ml/da) had the highest values. The excess

compared to the controls was by 22.2 and 30.6%, respectively. In the second experimental year, the foliar application of Phosphorus humate (300 ml/da) had the highest impact on the dry mass amount.

The treatment with humate fertilizers increased the percentage and species share of useful grasses and legumes in the natural grass stand from the first to the third experimental year, and the amount of motley grasses decreased by from 25.0 to 34.4% (Figure 2, 3 and 4).

In the first year of the experiment, grasses (*Agrostis capillaris*, *Bothriochloa ischaemum*, *Chrysopogon gryllus*, *Festuca ovina*), legumes (*Trifolium campestre*, *Lotus corniculatus*) and motley grasses took 12.4%, 3.0% and 84.6% of the total natural grass stand, respectively.

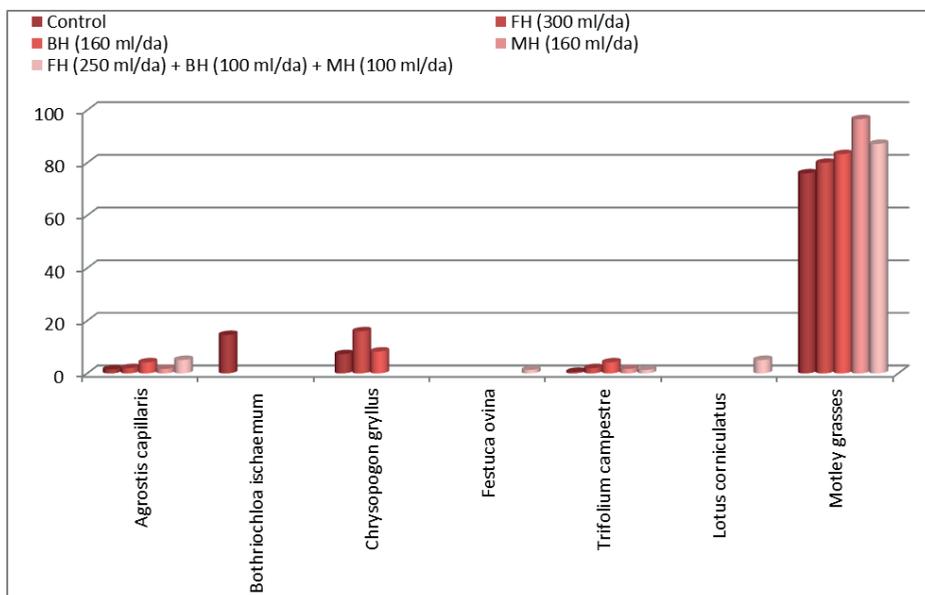


Figure 2. Botanical composition of a natural meadow of *Chrysopogon gryllus* type, treated by humate fertilizers in the first experimental year (2013)

Grasses in the treated variants occupied a smaller share in the composition of the grass mass compared to those in the control.

In contrast, legumes in fertilizer variants occupied from 1.7% (Molybdenum humate 160 ml/da) to 6.4% (Phosphorus humate - 250 ml/da + Boron humate - 100 ml/da + Molybdenum humate - 100 ml/da) of harvested from grass stand, at 0.6% - for untreated plots.

In the second experimental year, the percentage of grasses (*Agrostis capillaris*, *Bothriochloa*

*ischaemum*, *Chrysopogon gryllus*, *Festuca ovina*, *Anthoxanthum ododratum*, *Holcus lanatus*, *Cynosurus cristatus*) and legumes (*Trifolium campestre*, *Lotus corniculatus*) was higher with 11.4 and 23.0%, and the share of motley grasses decreased by 34.4%.

Grasses prevailed by 44.3% compared to the control only in the variants treated with Boron humate (160 ml/da).

The foliar application of Phosphorus humate (300 ml/da) and the combination of humate

fertilizers (Phosphorus humate - 250 ml/da + Boron humate - 100 ml/da + Molybdenum humate - 100 ml/da) positively affected the

share of legumes and increased their share in grass stand respectively from 7.0 to 75.9%.

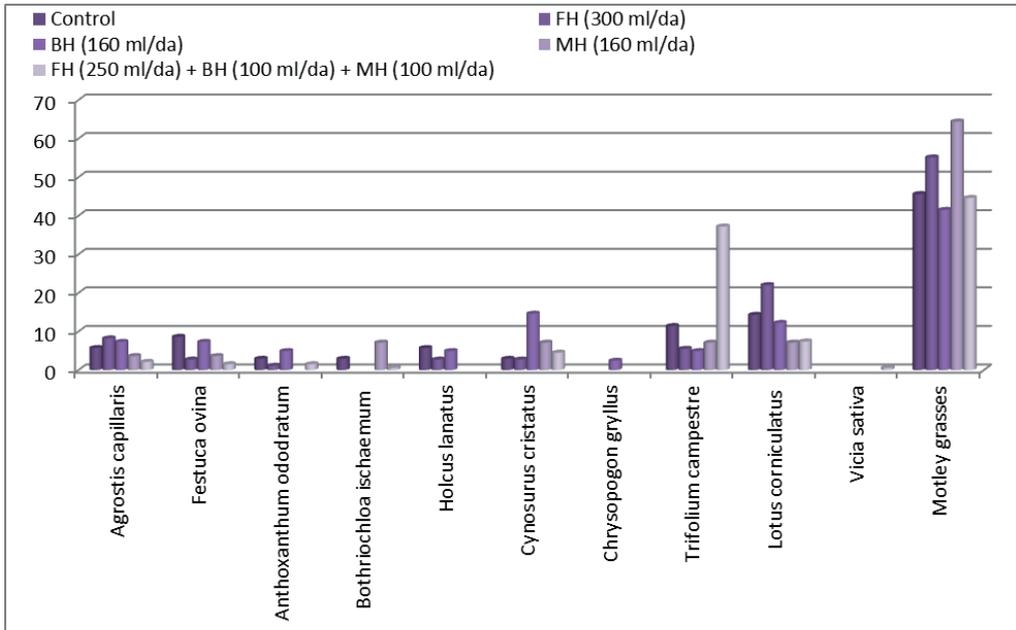


Figure 3. Botanical composition of a natural meadow of *Chrysopogon gryllus* type, treated by humate fertilizers in the second experimental year (2014)

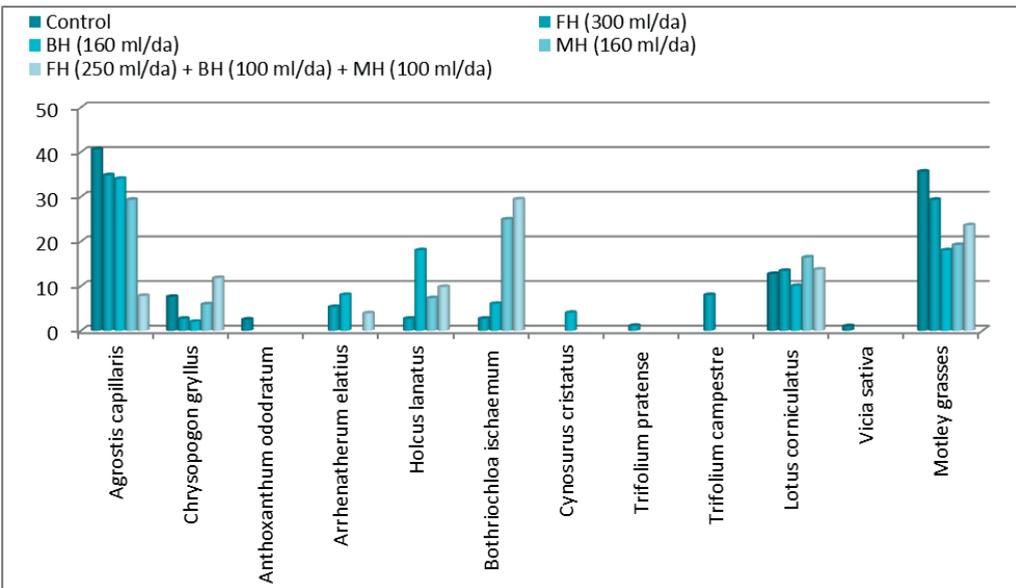


Figure 4. Botanical composition of a natural meadow of *Chrysopogon gryllus* type, treated by humate fertilizers in the third experimental year (2015)

In the third experimental year, positive changes were observed in the grass community. As a result of the imported fertilizers, a species diversity of grasses was found in all test variants. The characteristic species defining the grass stand *Chrysopogon gryllus* was displaced by *Bothriochloa ischaemum* and *Agrostis capillaris*, a trend, which continued from the previous year. The highest percentage share of *Agrostis capillaris* (40.6%) was registered in the non-treated control and the lowest (7.8%) in the variant with combined action of humate fertilizers. When applying Phosphorus humate (300 ml/da) the percentage share of *Agrostis capillaris* - 34.8% of the total species composition of the grass stand. The high presence of *Agrostis capillaris* in natural grasses determines a high pastoral value on grass stand (Andreoiu et al., 2020). *Bothriochloa ischaemum* prevailed most in the variants with Molybdenum humate (24.9%) and the combination of Phosphorus humate - 250 ml/da + Boron humate - 100 ml/da + Molybdenum humate - 100 ml/da (29.4%).

In the variants with application of Boron humate (160 ml/da), Phosphorus humate (300 ml/da), and the variants with a combination of humate fertilizers, the share of grass species, such as *Arrhenatherum elatius* was 8.0%, 5.3% and 3.9% respectively of the total grass stand composition.

The effect of foliar fertilization in all tested variants directly affected the legume component in the grass stand. Typical representatives are *Lotus corniculatus*, *Trifolium campestre*, *Trifolium pratense* and *Vicia sativa*. Their share is particularly pronounced in the variants fertilized with Phosphorus humate (300 ml/da), where their share reached 13.4%. This is the variant with the most balanced floristic composition, conditionally divided into groups (grasses:legumes:motley grasses) in the ratio 42.4:22.5:29.3%, which implies the formation of an above-ground mass with good nutritional value. The percentage of grasses and legumes in natural grass stands affects the quality of the aboveground mass (Gür & Şen, 2016; Francisquini Junior et al., 2020), and the manner and intensity of use, as well as the selective behavior of ruminants, lead to an increase in the density of reproductive and invasive species (respectively reduces the

volume of useful grass species preferred by farm animals) in the composition of meadow grasses (Naydenova & Mitev, 2011; Bayraktar, 2012; Tuna et al., 2013). The presences of highly productive species that have stabilizing properties dominate fertilized assemblages and enhance ecosystem stability (Yang et al., 2011). The applied fertilization improved and enriched the botanical composition of the natural grass stand, creating fodder that meets the needs of ruminants. In the third experimental year, the dominant species of legumes was *Lotus corniculatus*. Foliar fertilization with Molybdenum humate (160 ml/da) increased its percentage in the grass stand to 16.4%. In the group of clover, apart from *Trifolium campestre*, no other representatives characterizing this species have been identified (exception is var. 2, where the red clover participated with 1.1% of the total species composition).

## CONCLUSIONS

Foliar feeding of natural grass stand of *Chrysopogon gryllus*, type with organic biofertilizers based on humic acids affects the productivity of fresh and dry mass. Treatment with Molybdenum humate (160 ml/da) resulted in a significant ( $P < 0.01$ ) excess of fresh mass yield (570.0 kg/da) compared to the nontreated control (428.3 kg/da). The influence of other humate products is less pronounced, as the probable reason for this are the specific interactions between the specific climatic conditions during the year, the type of grass stand, the fertilization rate and the method of fertilizer application.

The effect of foliar treatment with biofertilizers had a significant effect on the floristic composition of the grass stand. The percentage and species share of the main biological groups (grasses, legumes, motley grasses) in the natural grass stand changed in a positive way from the first to the third experimental year. There is a reduced share of the main species *Chrysopogon gryllus* (characterizing the grass community) and motley grasses in the formed aboveground mass. The share of *Agrostis capillaris* and legumes (*Trifolium campestre* and *Lotus corniculatus*) significantly increased, which suggests better quality indicators of grass biomass.

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## THE INFLUENCE OF THE *Orobanche cumana* Wallr. ATTACK ON AN ASSORTMENT OF SUNFLOWER HYBRIDS UNDER BRAILA COUNTY CONDITIONS

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### Abstract

*In infested fields, Orobanche cumana Wallr. causes severe yields and quality losses. In this study, several commercial sunflower hybrids were analyzed. All the tested hybrids presented different levels of infestation, depending on the resistance genes of the used assortment. The hybrid Performer, without resistance genes, presented the highest attack rate, with an average attack rate of 3.09% and the lower attack rate was recorded in case of the hybrid LG 50.635 CLP, with an average attack rate of 0.19%. The highest production (kg/ha) was recorded for the SY BACARDI CLP hybrid, with an average yield per variant of 2493.4 kg/ha (tolerant to Orobanche cumana Wallr. up to breed E), and at the opposite pole, the highest production being registered for the hybrid LG 55.55 CLP, with 3235.3 kg/ha (resistant up to breed G). Regarding the percentage of oil, the lowest values have been recorded in case of hybrids ES JANIS and PERFORMER, with an oil percentage of 44.4%.*

**Key words:** *Orobanche cumana*, sunflower, attack degree, oil content.

### INTRODUCTION

Sunflower remains the main plant for obtaining oil in its cultivation area (Chiriac et al., 2018; Arghiroiu et al., 2015). The health status of sunflower culture, seed and oil production are affected by the presence of important pathogens and parasites (Mardare et al., 2015; Mardare et al., 2014; Cristea, 2005).

The yield stability and the agronomic and economic efficiency of sunflower cultivation depend on the influence of genotype, the level of applied technology, and the presence of an important number of diseases caused by the attack of parasitic pathogens. More than 30 diseases have been identified on sunflower (Gulya et al., 1994).

The parasite *Orobanche cumana* Wallr. lives attached on the sunflower's root system and it can cause severe damages that can reach up to 90% depending on the virulence of the populations and physiological races of the parasite in the area cultivated with sunflower and on the attack's intensity (number of broomrape plants formed on one sunflower plant) (Vranceanu, 2000).

Broomrape was described for the first time in Russia at the end of the 19<sup>th</sup> century. From the southern regions of Russia and Ukraine, the broomrape extended on the same time with the sunflower crops in the other riverside countries, Romania, Bulgaria and Turkey (Vranceanu, 2000).

If we talk about the current situation regarding the spread of broomrape around the world, in addition to Russia, Ukraine, Romania, Bulgaria, Turkey and Spain, as the main sunflower producers, we can also add Serbia, Moldova, Hungary, Greece, Tunisia, Israel, Iran, Kazakhstan, China, Mongolia and Australia because the parasite it has been also reported, and in a few other countries as well (Marinkovic et al., 2014; Gisca et al., 2013; Amri et al., 2012; Pacureanu-Joita et al., 2012; Burlov and Burlov, 2011; Dedic et al., 2009; Molinero-Ruiz et al., 2009).

The aim of the study was to identify the parasite population virulence (*Orobanche cumana* Wallr.) in Braila county and the influence on yield and oil content. These results are intended to offer information concerning the sunflower hybrids zoning in this area.

## MATERIALS AND METHODS

The testing methodology consisted in using an assortment of sunflower hybrids with known reaction against *Orobanche cumana* Wallr., to identify their virulence. The used hybrids are commercial and were described in trade catalogues by the producer regarding the reaction to the parasite. The research has been carried out over three years (2018, 2019, 2020), but the presence of this parasite was reported only in 2018 as the result of a natural infestation.

Being thought of as an experimental field with several factors, the observations regarding the attack of this parasite were made both in the variant considered as a control and in three other variants where solid and liquid fertilizers were applied. The trial design was randomized incomplete blocks, each variant having three repetitions.

**Factor 1 (the assortment of hybrids used):** H<sub>1</sub> (ES JANIS) - resistant to race G (Euralis); H<sub>2</sub> (MAS 92. CP) - resistant to race E (Maisadour); H<sub>3</sub> (SY NEOSTAR CLP) - tolerance to race E (Syngenta); H<sub>4</sub> (SY BACARDI CLP) - tolerance to race E (Syngenta); H<sub>5</sub> (LG 50.635 CLP) - resistant to race G (Limagrain); H<sub>6</sub> (LG 55.55 CLP) - resistant to race G (Limagrain); H<sub>7</sub> (PERFORMER) - without resistance genes (NARDI Fundulea); H<sub>8</sub> (FD-15 C27) - tolerance to races F-G (NARDI Fundulea); H<sub>9</sub> (ES GENESIS) - resistant to race G (Euralis).

**Factor 2 (fertilization):** unfertilized plot (control); urea fertilizer 46% N (90 kg/ha active substance); liquid fertilizer Last N (250 g/l N in one dose: 15 l/ha in 200 l water); liquid fertilizer Last N (250 g/l N in two doses: first application 15 l/ha in 200 l water and the second application, with the same dose, 14 days after).

Regarding the evolution of the parasite and the hybrids reaction, observations were made during the vegetation period. The frequency (F%), the intensity (I%) and the attacking rate (AR%) were evaluated at the end of flowering, by counting the parasite on each plant (Kaya Y. et al., 2004).

The frequency of the attack was calculated using the formula:  $F(\%) = \frac{N}{N_t} \cdot 100$ , where: N = the number of the infested sunflower plants; N<sub>t</sub> = the total number of analyzed plants.

The intensity of the attack is represented by the number of broomrape plants formed on one sunflower plant and was calculated using the formula:  $I(\%) = \frac{a}{N}$ , where: a = the number of *Orobanche cumana* Wallr. plants; N = the number of infested sunflower plants. Attack rate (AR) was calculated using the formula:  $AR(\%) = \frac{F(\%) \cdot I(\%)}{100}$ , where: F (%) = frequency, I (%) = intensity.

The percentage of oil was determined using the Infratec 1241 Grain Analyzer. To determine the percentage of seed oil, sunflower seeds were scanned using the infrared spectrometer for grain, Infratec 1241 Grain Analyzer, which is intended for whole seeds, using embedded infrared transmission technology to analyze a wide range of parameters (moisture, protein, oil, etc.)

## RESULTS AND DISCUSSIONS

Broomrape (*Orobanche cumana* Wallr.) is the primary parasite of sunflower in the SE area of Romania and it is especially spread over Constanta, Tulcea, Braila, Ialomița, Buzau and Calarasi counties, with a tendency of spreading toward the sunflower crops in the West of Romania (Pricop et al., 2011).

The broomrape's virulence increased significantly in the last two decades due to a short crop rotation and the use of non-resistant sunflower hybrids, causing a loss of yield and oil production (Pricop and Cristea, 2012).

Heavy infestations can cause >50% reduction in yields and severe quality losses (Duca and Glijin, 2013). Mean attack severity corresponds to susceptible cultivars (Terzic et al., 2010).

The parasite-host plant system studied in conditions of natural infestation depends on the homogeneity of the field infestation and on the genetic material had in the study.

The most important economic traits in sunflower are the productions obtained per hectare and the percentage of oil (Hladni et al., 2010; Marinković, 1992).

The experimental results were obtained in 2018 in Braila, Braila County. All the tested hybrids presented different levels of infestation with broomrape. The race structure was evaluated depending on the hybrids reaction and the results regarding the parasite frequency, intensity and attack rate are presented in Table 1.

Table 1. Results on the identification of *Orobanche cumana* Wallr. populations in 2018 in Braila

Experimental variants		Resistant genes	F (%)	I (%)	AR (%)
H <sub>1</sub> (ES JANIS)	unfertilized	resistant to race G	26.0	1.0	0.26
	urea fertilizer 46% N		24.7	1.0	0.25
	Last N (250 g/1 N in one dose)		25.9	1.7	0.44
	Last N (250 g/1 N in two doses)		12.5	0.3	0.04
			<b>Average</b>		<b>0.25</b>
H <sub>2</sub> (MAS 92. CP)	unfertilized	resistant to race E	100.0	2.7	2.70
	urea fertilizer 46% N		100.0	1.7	1.70
	Last N (250 g/1 N in one dose)		85.0	1.0	0.85
	Last N (250 g/1 N in two doses)		68.5	2.1	1.44
			<b>Average</b>		<b>1.67</b>
H <sub>3</sub> (SY NEOSTAR CLP)	unfertilized	tolerance to race E	50.0	1.4	0.70
	urea fertilizer 46% N		46.0	1.3	0.60
	Last N (250 g/1 N in one dose)		48.0	2.2	1.06
	Last N (250 g/1 N in two doses)		35.2	1.1	0.39
			<b>Average</b>		<b>0.69</b>
H <sub>4</sub> (SY BACARDI CLP)	unfertilized	tolerance to race E	60.0	1.2	0.72
	urea fertilizer 46% N		55.2	1.0	0.55
	Last N (250 g/1 N in one dose)		51.0	1.0	0.51
	Last N (250 g/1 N in two doses)		61.3	3.2	1.96
			<b>Average</b>		<b>0.94</b>
H <sub>5</sub> (LG 50.635 CLP)	unfertilized	resistant to race G	22.0	1.08	0.24
	urea fertilizer 46% N		20.1	0.9	0.18
	Last N (250 g/1 N in one dose)		23.2	0.7	0.16
	Last N (250 g/1 N in two doses)		20.2	0.8	0.16
			<b>Average</b>		<b>0.19<sup>b</sup></b>
H <sub>6</sub> (LG 55.55 CLP)	unfertilized	resistant to race G	33.0	1.1	0.36
	urea fertilizer 46% N		30.3	1.2	0.36
	Last N (250 g/1 N in one dose)		18.5	1.3	0.24
	Last N (250 g/1 N in two doses)		30.8	1.1	0.34
			<b>Average</b>		<b>0.39</b>
H <sub>7</sub> (PERFORMER)	unfertilized	without resistance genes	68.0	5.2	3.54
	urea fertilizer 46% N		65.9	4.1	2.70
	Last N (250 g/1 N in one dose)		72.2	3.8	2.74
	Last N (250 g/1 N in two doses)		78.9	4.3	3.39
			<b>Average</b>		<b>3.09<sup>†</sup></b>
H <sub>8</sub> (FD-15 C27)	unfertilized	tolerance to races F-G	68.0	3.6	2.45
	urea fertilizer 46% N		53.1	2.9	1.54
	Last N (250 g/1 N in one dose)		50.3	3.5	1.76
	Last N (250 g/1 N in two doses)		52.3	2.1	1.10
			<b>Average</b>		<b>1.71</b>
H <sub>9</sub> (ES GENESIS)	unfertilized	resistant to race G	21.0	1.2	0.25
	urea fertilizer 46% N		19.3	1.0	0.19
	Last N (250 g/1 N in one dose)		22.7	1.8	0.41
	Last N (250 g/1 N in two doses)		18.4	1.5	0.28
			<b>Average</b>		<b>0.28</b>

The parasite *Orobanche cumana* Wallr., has become more and more dangerous for sunflower crop in Romania. More than half of area cultivated with sunflower is infested with broomrape. The new populations of this parasite, very virulent, which are spread in areas situated near Black Sea, are changing their virulence in short time, the new sunflower hybrids which are resistant at the beginning of

their cultivation in this area, quickly loses their resistance (Pacureanu, 2014).

In the experimental year 2018, all the tested hybrids presented different levels of infestation, depending on the resistance genes of the used assortment (Table 1.). The hybrid Performer, without resistance genes, presented the highest attack rate, with an average attack rate of 3.09% and the lower attack rate was recorded

in case of the hybrid LG 50.635 CLP, with an average attack rate of 0.19%, it justifies this process, the hybrid having resistance genes up to the G breed.

Good results on the attack rate were also recorded in the case of hybrids, ES JANIS with an average attack rate of 0.25% and ES GENESIS, with an average attack rate of 0.28%, both having resistance genes up to the G breed. Pricop et al., 2011, identified race G and some populations more aggressive than the

race G, during a study carried during 2009-2010, in fields with sunflower monoculture at ARDS Valu lui Traian and Cogealac, located in Constanța County.

The infestation observed in the experimental field located in Braila, enables us to conclude that in our area a physiological race that is more aggressive than race G, exists (Table 2.), but cultivating hybrids with the corresponding gene, can prevent yield losses.

Table 2. Explanations concerning the identification of *Orobanche cumana* Wallr. races in the experimental field

Differential host	Field status
Hybrid without resistance genes	Heavily infested, the field partially blossomed
Hybrid resistant to race E	The plots were infested, the plants bloomed, but the size being smaller
Hybrid tolerant to race E, F, G	Average infestation, the plants bloomed, smaller size
Hybrid resistant to race G	Weakly infested

The high susceptibility of Clearfield cultivars to broomrape could be the consequence of a breeding process in account of the fact that as in Clearfield cultivars less attention is paid to genetic resistance to this parasite since broomrape could be chemically controlled in Clearfield crops (Kaya et al., 2012).

As it can be seen in Table 3., the highest production was recorded for the SY BACARDI CLP hybrid, with an average yield per variant of 2493.4 kg/ha (tolerant to *Orobanche cumana*

Wallr. up to breed E), and at the opposite pole, the highest production being registered for the hybrid LG 55.55 CLP, with 3235.3 kg/ha (resistant up to breed G). Regarding the percentage of oil, the lowest values have been recorded in case of hybrids ES JANIS and PERFORMER, with an oil percentage of 44.4%. The following hybrids stood out with an oil percentage of 45.1%, SY NEOSTAR CLP and FD-15 C27.

Table 3. Results concerning the influence of the *Orobanche cumana* Wallr. attack on sunflower medium production per hectare (kg/ha) and oil content (%), 2018

Experimental variants		Resistant genes	AR (%)	Medium production per hectare (kg/ha)	Oil content (%)
H <sub>1</sub> (ES JANIS)	unfertilized	resistant to race G	0.26	2913.1	44.2
	urea fertilizer 46% N		0.25	2857.4	44.4
	Last N (250 g/1 N in one dose)		0.44	3030.0	44.5
	Last N (250 g/1 N in two doses)		0.04	3171.7	44.1
	<b>Average</b>		<b>0.25</b>	<b>2993.0</b>	<b>44.4</b>
H <sub>2</sub> (MAS 92. CP)	unfertilized	resistant to race E	2.70	2395.3	45.0
	urea fertilizer 46% N		1.70	2832.7	44.8
	Last N (250 g/1 N in one dose)		0.85	2953.7	45.1
	Last N (250 g/1 N in two doses)		1.44	2858.0	44.9
	<b>Average</b>		<b>1.67</b>	<b>2759.9</b>	<b>45.0</b>
H <sub>3</sub> (SY NEOSTAR CLP)	unfertilized	tolerance to race E	0.70	2293.5	45.1
	urea fertilizer 46% N		0.60	2992.3	45.2
	Last N (250 g/1 N in one dose)		1.06	2382.0	45.0
	Last N (250 g/1 N in two doses)		0.39	3110.0	44.9
	<b>Average</b>		<b>0.69</b>	<b>2694.5</b>	<b>45.1</b>
H <sub>4</sub> (SY BACARDI CLP)	unfertilized	tolerance to race E	0.72	1575.1	45.2
	urea fertilizer 46% N		0.55	2806.7	45.1
	Last N (250 g/1 N in one dose)		0.51	2812.3	45.0
	Last N (250 g/1 N in two doses)		1.96	2779.7	44.8
	<b>Average</b>		<b>0.94</b>	<b>2493.4↓</b>	<b>45.0</b>

H <sub>5</sub> (LG 50.635 CLP)	unfertilized	resistant to	0.24	2922.0	44.3
	urea fertilizer 46% N	race G	0.18	3033.0	44.5
	Last N (250 g/1 N in one dose)		0.16	2913.7	44.5
	Last N (250 g/1 N in two doses)		0.16	3351.0	44.7
<b>Average</b>			<b>0.19<sup>↓</sup></b>	<b>3054.9</b>	<b>44.5</b>
H <sub>6</sub> (LG 55.55 CLP)	unfertilized	resistant to	0.36	3217.2	45.2
	urea fertilizer 46% N	race G	0.36	3206.7	45.0
	Last N (250 g/1 N in one dose)		0.24	3273.0	44.7
	Last N (250 g/1 N in two doses)		0.34	3244.3	44.6
<b>Average</b>			<b>0.39</b>	<b>3235.3<sup>↑</sup></b>	<b>44.9</b>
H <sub>7</sub> (PERFORMER)	unfertilized	without	3.54	2801.6	44.2
	urea fertilizer 46% N	resistance	2.70	3177.0	44.5
	Last N (250 g/1 N in one dose)	genes	2.74	2617.0	44.7
	Last N (250 g/1 N in two doses)		3.39	2878.7	44.3
<b>Average</b>			<b>3.09<sup>↑</sup></b>	<b>2868.6</b>	<b>44.4</b>
H <sub>8</sub> (FD-15 C27)	unfertilized	tolerance to	2.45	2283.3	45.3
	urea fertilizer 46% N	races F-G	1.54	2403.7	44.9
	Last N (250 g/1 N in one dose)		1.76	2398.3	45.0
	Last N (250 g/1 N in two doses)		1.10	2996.0	45.2
<b>Average</b>			<b>1.71</b>	<b>2520.3</b>	<b>45.1</b>
H <sub>9</sub> (ES GENESIS)	unfertilized	resistant to	0.25	3027.9	44.3
	urea fertilizer 46% N	race G	0.19	3624.3	44.6
	Last N (250 g/1 N in one dose)		0.41	3180.7	44.5
	Last N (250 g/1 N in two doses)		0.28	2937.3	44.2
<b>Average</b>			<b>0.28</b>	<b>3192.6</b>	<b>44.4</b>

## CONCLUSIONS

The study proved that the parasite is present in the territory, the populations of *Orobanche cumana* Wallr. being aggressive. All the tested hybrids presented different levels of infestation, depending on the resistance genes of the used assortment. The hybrid Performer, without resistance genes, presented the highest attack rate, with an average attack rate of 3.09% and the lower attack rate was recorded in case of the hybrid LG 50.635 CLP, with an average attack rate of 0.19%.

The highest production was recorded for the SY BACARDI CLP hybrid, with an average yield per variant of 2493.4 kg/ha (tolerant to *Orobanche cumana* Wallr. up to breed E) and at the opposite pole, the highest production was registered for the hybrid LG 55.55 CLP, with 3235.3 kg/ha (resistant up to breed G).

Regarding the percentage of oil, the lowest values have been recorded in case of hybrids ES JANIS and PERFORMER, with an oil percentage of 44.4%.

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## THE IMPACT OF THE ENTOMOFAUNA ON THE PLANTS OF *Phacelia tanacetifolia* Benth. IN THE COLLECTION OF THE “AL. CIUBOTARU” NATIONAL BOTANICAL GARDEN (INSTITUTE)

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### Abstract

*The species P. tanacetifolia has high potential due to its honey, ornamental and forage value, with special properties that make it advantageous to be grown and used in beekeeping, under the environmental conditions of the Republic of Moldova. It has been considered a plant with attractive flowers for honeybees and all the associated entomofauna. Under the climatic conditions of RM, it attracts a large range of insects, with various trophic spectra, so that pollinating species predominate in the flowering stage, which is staggered (40-55 days out of a total of 104 days of the growing season) and provides a stable and long-lasting source of food for melliferous insects. The phytosanitary monitoring carried out established the presence of a significant complex of insects, represented by 23 species included in 6 orders and 17 families. The species of the order Coleoptera (7) were predominant, followed by Hymenoptera (6), Diptera (4), Hemiptera (3), Lepidoptera (2) and Homoptera (1 species). The diversity and numerical density was higher in species of bumblebees and honeybees, as specialized pollinators of flowering plants, which visited the flowers throughout the flowering period.*

**Key words:** *Phacelia tanacetifolia, entomofauna, trophic specialization.*

### INTRODUCTION

Currently, agriculture practiced in different ways, for example in private, associative or state farms, needs more research and implementation of its results and a wider range of knowledge, to be able to cope with the many issues that arise in connection with the cultivation technologies, especially concerning newly introduced plant species (Roșca et al., 2011). The research on the cultivation of new plants, especially species with high potential for honey production and that can be used as natural fertilizers for degraded soil in anthropogenic agrocenoses, can play an important role by elucidating and applying modern methods concerning the ecological and phytosanitary impact of terrestrial insect complexes on new species of honey plants (Busuioc, 2004, Roșca, et al., 2011).

Internationally, in the European Union, research on pollinating insects has been conducted in scientific centres, focusing on the effectiveness of the application of a series of beneficial measures for monitoring pollinating

entomofauna in order to protect natural and anthropogenic habitats, to offer scientific and applied support to beekeeping and to set restrictions on the excessive chemical management in the protection system of anthropogenic agrocenoses (Talmaciu, 2014, Зауралов, 1985).

The bibliographic review on the respective topic highlighted the presence of over 1000 species of honey plants, with various classifications according to such criteria as: morpho-taxonomic and bioecological criteria, potential for beekeeping, economic-productive efficiency in obtaining biological production and honey per hectare (Pîrvu, 2000, Eremia, 2014). The research on entomofauna has resulted in the estimation of various determinations and ways of formation of insect associations with different trophic specialization and adaptation to plants, as well as the elucidation of harmful and beneficial species and the balance in the system host plant - harmful organism - environmental factors (Perju, 1995; Busuioc, 2004).

Natural populations of entomophagous insects play an important role in regulating the numerical density of pests, but their frequency is insufficient for a reliable protection of crops. In such situations, a recommended biological protection measure is the additional release of useful predatory insect species in order to reduce the abundance of parasitic pests in anthropogenic agro-ecosystems (Perju, 1995, Volosciuc, 2014).

Based on the literature review, we have estimated the significant role of insect complexes on plants, where some species of predatory-omnivorous insects are used as living biological agents in combating other species of harmful insects, which are characteristic of crop plants. We would also like to mention the complex harmful effect of parasitic species that cause considerable damage to agricultural crops, being also vectors of phytopathogenic bacteria (Tălmăciu, 2014).

Our research has been conducted on *Phacelia tanacetifolia* Benth. - a species in the family Hydrophyllaceae Lindl. (=Boraginaceae Juss.), the genus *Phacelia* (=Eutoca), which includes over 150 species of plants occurring in the wild flora of the American continents (USA to the Andes Mountains, Chile) (Cherniavskih, 2018, Чибиc, 2017). It has been known since 1832 and was brought to Europe from North America by the naturalist researcher David Douglas (Чибиc, 2017).

Lacy phacelia, also known as purple tansy, is an annual herbaceous plant with multiple uses and can play an important role for natural and anthropogenic biocoenoses under the environmental conditions of the Republic of Moldova. In the NBGI, the variety 'Melifera' was created and approved and it was registered in the Catalogue of Plant Varieties of the Republic of Moldova in 2014. In 2016, the Plant Variety Patent no. 206 / 2016.05.31 was obtained. During our previous research, the chemical composition of the 'Melifera' cultivar of lacy phacelia was determined: ADF - 30.2%, NDF - 53.4%, ADL - 7.0%, cellulose - 23.2%, carbon - 48.1%, potassium - 36.8 g/kg, calcium - 43.6 g/kg, zinc - 32.8 mg/kg, organic substances - 866 g/kg (Țiței, 2017; 2019). It is remarkable that lacy phacelia possesses melliferous and productive capacities and up to 300 (556), (600-100) kg/ha can be obtained

(Cherniavskih, 2018; Ion et al., 2018; <http://www.eurohonig.com>). The nectar of *P. tanacetifolia* contains organic substances in large quantities such as: sucrose (53.2 %), glucose (14.7 %) and fructose (21.9 %) (Зауралов, 1985), besides, the amount of nectar produced by this crop in the flowering stage is also an efficient nutritional source for 60 species of parasitoids and other species of various trophic specializations (Brown, 2001).

The purpose of our research was to carry out evidence and inventory studies on the impact of plants of the species *P. tanacetifolia* on the entomofauna under the influence of the climatic conditions of the Republic of Moldova. We set the following objectives: to determine the frequency diversity of the insect species identified in various phenological stages on the lacy phacelia plants; the elucidation of the spectrum of trophic specialization and the classification of insects according to this criterion and their taxonomic position.

## MATERIALS AND METHODS

The research was done in the experimental sectors of the National Botanical "Al. Ciubotaru" Garden (Institute) (NBGI), 46°58'25.7" latitude and N28°52'57.8" longitude, in demonstration plots, where a wide range of plants with multiple uses has been cultivated annually and extensive research on their morpho-biological features and melliferous capacity, forage quality and efficiency in restoring the structure and fertilization of damaged soil has been conducted. The experimental plots have different areas, depending on the research criterion (10 m<sup>2</sup>, 100 m<sup>2</sup>) (Figure 1). The plants of the 'Melifera' cultivar of *Phacelia tanacetifolia* Benth. served as research subjects. The seeds were sown in March, at a depth of 2-3 cm; the distance between rows was 15 cm and the seeding rate 0.6 g/m<sup>2</sup>.

Seasonal and monthly surveys were conducted in 2019-2020, during different phenological stages of the plants (spring-summer), through manual collecting with the insect net, visual observations and findings with the digital camera, with the purpose of determining the diversity of insects on the plants of *Phacelia tanacetifolia* Benth. and the nature of their interaction.

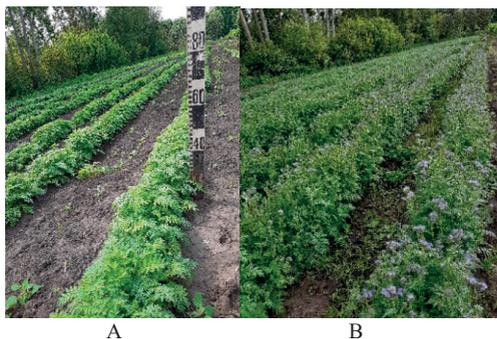


Figure 1. A - experimental plot planted with *P. tanacetifolia*; B - lacy phacelia in the flowering stage

Over 150 samples of plants and insects collected from various parts and organs of the plants, influenced by the environmental conditions in the respective stages, were analyzed. Subsequently, laboratory analyzes were performed to identify the morphobiological features of the insects, their taxonomic affiliation and trophic specialization, confirmed by descriptions, pictures, documentation, studying guides for determining insect species, monographs, manuals and other literature on general, special and applied entomology (Бей-Биенко, 1966; Плавильщиков, 1994; Perju, 1995; Busuioc, 2001; Cozari, 2010; Talmaciu, 2014).

## RESULTS AND DISCUSSIONS

Extensive research on the non-traditional plant species has been conducted at global and regional level, including the Republic of Moldova, aiming at introducing and breeding this new plant species with multiple uses, by bioecological adaptations, obtaining raw material for soil fertilization, fodder and a significant source of pollen and nectar for beekeeping. In this context, we would like to mention the importance of *P. tanacetifolia*, used and researched as a promising species that possesses productive and bioecological qualities appreciated in the agro-economy of the Republic of Moldova.

As a result of the morpho-taxonomic and bioecological research conducted in the NBGI, lacy phacelia was described as an annual herbaceous species, with the growing season of about 104 days and the flowering phase lasting 45-55 days, being initially investigated as a

fodder crop, and then, after its successful adaptation to the environmental conditions, other valuable qualities were noticed, such as its melliferous potential and positive impact on soil fertility. So, the species is currently characterized as a nectar-pollen producing crop and a source of nutrition for pollinating insects dominated by honey-producing species and those of high ecological importance.

These plants stay attractive for a long time for all spontaneous terrestrial entomofauna with different trophic spectrum, noticed especially in the flowering stage, because the plants produce a large number of inflorescences, and the flowering occurs in acropetal order, is staggered, with fragrant distinct smell, thanks to the compact nectariferous disc at the flower base. Honey bees, due to their abundance and frequency, predominate the persistence area over the flowers and are also the main pollinators that help the formation of the effective mass of flowers and seeds. A study shows an increase in the number of seeds on average by 24 %, in the number of flowers in inflorescence - by 1.4 times, and their presence was observed throughout the growing season (Васильева, 2017).

Our studies indicate the start of the growing season with the seeds being sown in early March, followed by germination and formation of vegetative and generative organs until mid-April-May, depending on environmental factors. The plants are erect, branched, producing up to 20-25 shoots, with lateral branches, the stem is juicy-fibrous, the leaves - alternate, sessile, pinnately-lobed, green and with shades of blue. The inflorescences are fan-shaped, 15-20 on each plant, consist of up to 70 flowers on the main shoots and up to 30-40 flowers on the lateral ones. The inflorescences consist of 4-8 one-sided coiling cymes (curls), with 18-22 flowers each (Figure 2).



Figure 2. Aspects of the inflorescence of *P. tanacetifolia*

The flowers are bell-shaped, of blue-lavender colour, with attractive scent and a lifespan of up to 2 days.

At the same time, monitoring surveys were carried out to identify the entomofauna present in the experimental plot during the growing season and its interaction with the lacy phacelia plants. Insects and invaded plant organs were sampled for further analysis in the laboratory.

Visually and microscopically, we determined some aspects related to the morpho-taxonomic characteristics, preparing some classifications

of the trophic spectrum of the insects from the samples taken from various parts and organs of plants. As a result of this preliminary research on entomofauna and on the impact of environmental conditions on it (2019-2020), 23 species of terrestrial insects were identified in various stages of development of *P. tanacetifolia* plants, taxonomically belonging to 17 families and 6 orders, estimated in Table 1, where the classification of the trophic specialization was also given.

Table 1. The diversity of entomofauna in the plantations of *P. tanacetifolia* and the systematization of the taxonomic units classified according to the trophic spectrum

No	Order	Family	Species	Trophic spectrum
1	Hymenoptera	Apidae	<i>Apis mellifera</i> (Linnaeus, 1758)	Pollen and nectar
2			<i>Bombus terrestris</i> (Linnaeus, 1758)	Pollen and nectar
3			<i>B. lapidarius</i> (Linnaeus, 1758)	Pollen and nectar
4			<i>B. hortorum</i> (Linnaeus, 1761)	Pollen and nectar
5		Halictidae	<i>Lasioglossum malachurus</i> (Kirby, 1802)	Pollen and nectar
6		Megachilidae	<i>Xylocopa valga</i> (Gerstaecker, 1872)	Pollen and nectar
7	Hemiptera	Coreidae	<i>Tritomegas bicolor</i> (Linnaeus, 1758)	Phytophagous, nectariphagous
8		Rhopalidae	<i>Corizus hyoscyami</i> (Linnaeus, 1758)	Phytophagous
9		Miridae	<i>Liocoris tripustulatus</i> (Fabricius, 1781)	Omnivorous
10	Homoptera	Cicadidae	<i>Cercopis arcuata</i> (Fieber, 1844)	Phytophagous
11	Coleoptera	Cerambycidae	<i>Agapanthia violacea</i> (Fabricius, 1775)	Phytophagous
12		Mordellidae	<i>Mordella aculeata</i> (Linnaeus, 1758)	Phytophagous
13		Coccinellidae	<i>Coccinella septempunctata</i> (Linnaeus, 1758)	Zoophagous, phytophagous Entomophagous, aphidophagous
14		Scarabaeidae	<i>Epicometis hirta</i> (Poda, 1716)	Phytophagous, nectariphagous
15			<i>Valgus hemipterus</i> (Linnaeus, 1758)	Phytophagous
16			<i>Cetonia aurata</i> (Linnaeus, 1758)	Phytophagous
17		Chrysomelidae	<i>Cryptocephalus sericeus</i> (Linnaeus, 1758)	Phytophagous
18	Diptera	Syrphidae	<i>Syrphus pyrastris</i> (Linnaeus, 1758)	Pollen, plant liquids, zoophagous
19			<i>S. ribesii</i> (Linnaeus, 1758)	Pollen, plant liquids, zoophagous
20		Sarcophagidae	<i>Sarcophaga carnaria</i> (Linnaeus, 1758)	Plant liquids
21		Tachinidae	<i>Xylota segnis</i> (Linnaeus, 1758)	Plant liquids
22	Lepidoptera	Satyridae	<i>Lasiommata maera</i> (Linnaeus, 1758)	Nectariphagous
23		Lycaenidae	<i>Polyommatus icarus</i> (Rottenburg, 1775)	Nectariphagous

These were significant results for the identification of species that are efficient nectar-feeding pollinators. At the same time, we would like to mention the presence and the predominance of honey-producing species belonging to the Apidae family (*Apis mellifera*, species of *Bombus*, *Lasioglossum malachurus*, *Xylocopa valga*) (Figure 3).

The collected material, which was morphologically analyzed and the taxonomic affiliation was established, was subsequently classified based on the obtained results to estimate the comparative share (%), in systematic terms, according to the orders,

density and abundance of the determined insect species.



Figure 3. A - the species *X. valga*; B - *B. terrestris* found on the flowers of *P. tanacetifolia*

The obtained ratio reflects the presence of insects according to their orders: Coleoptera - 7 species (31%), followed by Hymenoptera - 6 species (26%), Diptera - 4 species (17%), Hemiptera - 3 species (13%), Lepidoptera - 2 species (9%), and the order Homoptera (4%) was represented by only one species (Figure 4). Adult individuals predominated in insect associations; most of them were phytophagous, preferentially feeding on nectar and flower pollen, mixed with liquids, exudates, dew and other plant metabolic products useful for the vitality of insects that play an important role as pollinators and honey producers. The subsequent classification according to the trophic spectrum, showed that the group of phytophagous insects prevailed (91%), which denotes their preference for *P. tanacetifolia* and

their adaptation to these plants due to the nutrition sources they provide, creating favourable conditions for their reproduction, and the ecological-biological efficiency of the interaction between the insects and the host plant.

At the same time, the omnivorous-zoophagous insect species were significantly less numerous, their share being only 9 % of the total number of analyzed species. For us, the most important result of our research is the abundance and the harmonious interaction between phytophagous insects and the host plants, as an important qualitative factor for the maintenance of phytocenoses, honey yields and production of high quality seed material. This interaction depends on the impact of insects and the response of plants (Танский, 1988).

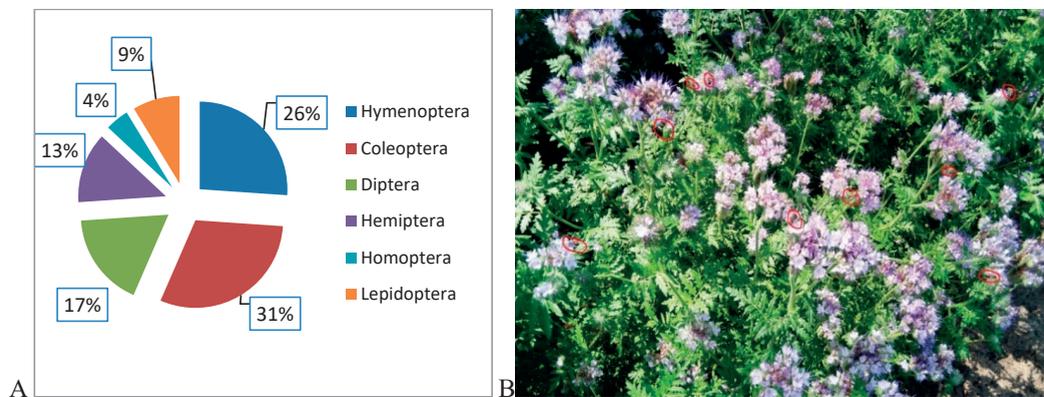


Figure 4. A - Comparative share of insect species according to orders; B - *P. tanacetifolia* in the flowering stage visited by pollinating insects

Another important finding was the establishment of the harmful impact of invasive species that partially affected the plants during some periods of the growing season, when phytoparasitic colonies of aphids (*Aphis* sp.) and thrips (*Thrips* sp.), which also occurred on plants, already as harmful species, were the most frequently and abundantly detected. In addition, we would like to mention the significance of another insect species with double trophic spectrum – *Epicometis hirta* (apple blossom beetle), detected in the flowering stage as a pollinator, but also as a pest of the developing fruits and seeds. In this context, on plants, there was a struggle for existence determined by the nutritive factor, between the associated complexes of predatory-

omnivorous (entomophagous) insects and the phytoparasitic pests, thus, the latter were devoured by entomophages that regulated the density of their populations, as a means of natural biological protection of *P. tanacetifolia* plants, making it possible to exclude pesticides totally.

Extensive analyses and evaluations of that study also established the comparative correlation between groups of parasitic insects (those with sucking-stinging and chewing mouthparts) in impact with nectarophagous species, which by their frequency and abundance dominate plants and intervene as nectarophagous pollinators that do not affect the plants and are significant for the researched

species and for other new honey-fodder species for the Republic of Moldova.

During the entire flowering stage and during the day (8:30-18.00), the most active and abundant species observed on plants were *Apis mellifera* L. and the bumblebees *Bombus terrestris* L., *B. lapidarius* L., *B. hortorum* L., regardless of the weather conditions (sunny, cloudy and even drizzle). However, at noon, from 11:30 to 12:30, they were the most active and productive. We estimated that the productive efficiency of a honeybee was on average 4-12 seconds on an inflorescence, depending on the size of the inflorescence, and of a bumblebee 8-10 seconds, and up to 5-9 insects could be simultaneously present on a flowering plant.

According to the research conducted by Vasileva (2017), in the lacy phacelia plantations, along with the honeybees (2 individuals/m<sup>2</sup>), there were other species of pollinating insects, which were not detected by us in the collection of the NBGI, such as *Myatropa florea* L. (1.6 individuals/m<sup>2</sup>), *Vespula vulgaris* L. (1 individual/m<sup>2</sup>) *Bombus agrorum* L. (1 individual/m<sup>2</sup>) etc.

In conclusion, we mention that the preliminary study has estimated the bioecological and productive significance of the insect complexes associated with the species *P. tanacetifolia* and the trophic relationships between them, determining the advantageous aspects that can be useful for beekeepers.

## CONCLUSIONS

Based on the research carried out at the "Al. Ciobotaru" National Botanical Garden (Institute) on the species *P. tanacetifolia* as a crop with multiple utility, with melliferous qualities, the impact of the entomofauna on the researched plants was assessed by determining the indices of diversity, density and the trophic specialization of the researched insect complexes.

As a result of the research, insect associations belonging to 6 orders, 17 families and 23 species have been detected on *P. tanacetifolia* plants during the entire growing season. The estimated species diversity was classified comparatively according to the prevalence of insects on plants by order and trophic

specialization, concluding that 91% were phytophagous species, while only 9% were omnivorous-zoophagous species. This fact indicates the preference of insects for the studied plant species and demonstrates the high potential of *P. tanacetifolia* as a honey plant, significant for the entire pollinating fauna and of interest to beekeepers.

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## THE INFLUENCE OF WORM COMPOST ON QUALITY AND HARVEST OF SOME FORRAGE CROPS

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### *Abstract*

*This article synthesizes own experience in the bioconversion of organic waste through worm cultivation. The main objective of the research was to assess the development of organic agriculture, using the technology of worm cultivation in the bioconversion of biodegradable waste in order to obtain the organic fertilizer (the worm compost) and ecological agricultural production. The relevant elements of the research consisted in estimating the role of hybrid species of earthworms Red California (*Eisenia foetida andrei*) in the bioconversion of biodegradable organic waste and obtaining the organic fertilizer (the worm compost), main element for the development of organic agriculture. As a result of researches it was found, that the values of quality indicators of the worm compost exceeded the essentially those of the nutritive substrate, and the incorporation of the worm compost in the soil, at a dose of 4 t/ha, has diminished the period of germination and ripening of forage crops, increasing harvest from 64.92 up to 110.14% and improved their quality, through diminishing nitroso compounds content.*

**Key words:** bioconversion, biodegradable organic waste, worm compost, forage crops, quality.

### INTRODUCTION

Obtaining organic agricultural products in recent years occupies a special place in the development of modern organic agriculture. The development of organic agriculture implies a return to the values of traditional agriculture, but not to its methods. The process and procedures for obtaining organic products are regulated by strict production rules and principles, which start from the quality, that the soil must possess and until the actual obtaining of the final product. Organic farming is carried out directly by using organic fertilizers, controlling diseases and pests through integrated ecological methods, total exclusion from agricultural technologies of industrially obtained chemicals (fertilizers, pesticides, growth stimulants) and completely giving up genetic engineering applications.

The ecological situation in the Republic of Moldova, for the last 4-5 decades of the XX century, has worsened as a result of the capitalization, practically total, of the territory and under the influence of decreasing the amount of humus in the soil. From this point of view, the territory of the republic can be considered an area with ecological balance

disorders. The ecological status is largely marked by the result of the socio-economic policy promoted in the post-war period in the USSR. The territory of the Republic of Moldova often served as an experimental base for: testing various chemicals (pesticides, herbicides and mineral fertilizers); implementation of intensive irrigation and the formation of gigantic zootechnical complexes (Băbălău et al., 1991), which had serious consequences for the environment.

At present, in order to solve the ecological problems of the environment, worldwide, modern methods and technologies are developed, of perspective for the technology of bioconversion of organic waste. In order to avoid the pollution of the asbestos environment and to obtain profit from the use of organic waste in many states (USA, Japan, Italy, Hungary, France, Ukraine, etc.) is practiced the processing by worm cultivation of biodegradable organic waste (Boclaci & Cremeneac, 2011; Cremeneac et al., 2012; Gorodnyi et al., 1996).

The objectives of the carried out researches consisted in the use of bioconversion technology for organic waste by worm cultivation for solving important ecological

problems: complete bioconversion of organic waste; improving and reviving soil fertility; obtaining long-acting ecological organic fertilizers; increasing the harvest of agricultural crops; environmental protection.

The technology of processing organic waste by this method is based on the biological capacity of the earthworms to use the solid fraction of organic waste as a substrate and source of nutrients. In a relatively short period of time, the valuable organic fertilizer (worm compost) can be obtained. The obtained worm compost has a high biological activity, in which are concentrated significant amounts of macro- and microelements, growth stimulants, antibiotics, vitamins and others. Its use at a dose of 3-10 t / ha contributes to increasing productivity and improving the quality of agricultural crops (Boclaci & Cremeneac, 2013; Cremeneac & Boclaci, 2015; Kosolapova et al., 1996).

The technology of bioconversion of organic waste through worm cultivation is a perspective method, which can be practiced in all types of households (public, peasant and private). It provides for the use of organic animal, household and vegetable waste, which has been partially subjected to the fermentation process, as a nutrient substrate and living environment for worm culture (Povkhan et al., 2004).

The research results demonstrate the importance of worm compost as an organic fertilizer, for the physiological development of plants, increasing the harvest and improving the quality of the obtained products, thus favoring the development of organic agriculture.

## MATERIALS AND METHODS

The experiment was organized in the Technological-Experimental Station "Maximovca", where in five sectors for worm cultivation, with a width of 1m and a length of 50 m was placed the nutrient substrate prepared for bioconversion through worm cultivation.

The period of the experiment depended on the physiological development of the plants.

In order to obtain forage crops it was organized experiment in field conditions in which were included three types of forage crops: alfalfa varieties 'Tuna', fodder beet variety 'Ekkendorfskaya' and maize varieties 'M-450'. Surface lots amounted to 2 acres. For each

fodder crop were used three lots: one control and two experimental. For lot I was kept natural background, lot II was fertilized with worm compost (from considerations 4 t/ha), and the third lot - ammonium nitrate (285 kg/ha), according to the scheme of the experiment (Table 1).

Before the incorporation of fertilizers in the soil, was performed the soil preparation (autumn - tilling to a depth of 30 cm- 40 cm and spring - loosening (by harrowing). Fertilization was carried out in early spring, immediately after snow melting on the autumn plowing. After harvesting of the forage crops, was carried out the determination of some quality indicators in accordance with the usual methods (Popov A. et al., 1973; Razumov V. et al., 1986).

Table 1. The experimental scheme

The lots	The conditions of the experiment	Investigations carried out
I - control	Natural background	Will be determined: a) observations on the physiological development of fodder crops b) the quality of worm compost and fodder crops; c) harvest for each fodder crops
II - experimental	With worm compost background (4 t / ha)	
III - experimental	With ammonium nitrate background (250 kg/ha)	

Materials for research were used the manure of cattle, subjected to the fermentation process for 6-7 months, subsequently used as a nutrient substrate, obtained worm compost, corn, alfalfa and fodder beet, and the object of research - earthworm California Red Hybrid, biotransformer of organic waste.

The nutrient substrate, placed in the sectors for worm cultivation, was subjected to biochemical analysis and to the determination of its nutritional value. The quality of the nutrient substrate (initially and during the experiment) and of the obtained fertilizer as a result of the bioconversion of organic waste (worm compost) was determined according to the following indicators: active acidity (pH), amount of ammonia, total nitrogen, organic matter, potassium, calcium, magnesium, phosphorus and non-pathogenic bacterial flora. The biochemical analysis of the quality of the nutrient substrate, of the obtained fertilizer and the fodder crops was performed according to the usual methods, set out below: active acidity,

using the pH meter; total nitrogen - according to the Kjeldahl method (Popov, et al., 1973); the ammonia content - according to methodological guidelines (Poloz et al., 1987), and the calcium, phosphorus, and potassium content - according to the methods from the handbook of Razumov et al. (1986).

## RESULTS AND DISCUSSIONS

In the sectors for worm cultivation, 25 tons of organic waste were placed, which were subjected to bioconversion by worm culture in order to obtain the organic fertilizer – worm-compost.

Previously, the organic waste was subjected to the fermentation process for 6 months. After fermentation it was placed the nutrient substrate with a thickness of 25-35 cm - in summer and 35-45 cm - in winter in the sectors for worm cultivation. Each sector for worm cultivation was divided into sections with dimensions of 1 m × 2 m.

During a month, in order to reduce the ammonia content and regulate the active acidity, the nutrient substrate in the sectors was sprayed for a week - daily, and then once a week with drinking water. Subsequently, the substrate prepared for worm cultivation was subjected to chemical analyzes in order to determine its quality. As a result of the analyzes, it was found that the nutrient substrate according to its chemical composition can be used as a nutrient substrate and living environment for worm culture, in the process of bioconversion of organic waste by worm cultivation (Table 2).

Table 2. Quality indicators of the nutrient substrate

Quality indicators	Indicator values, M ± m
Active acidity (pH), conventional units	7.57 ± 0.08
Ammonia, mg/kg	5.56 ± 0.57
Total nitrogen, %	0.83 ± 0.63
Organic matter, %	30.35 ± 0.60
Magnesium, %	1.17 ± 0.52
Phosphorus (P <sub>2</sub> O <sub>5</sub> ), %	0.65 ± 0.32
Potassium (K <sub>2</sub> O), %	0.68 ± 0.01
Calcium, %	0.55 ± 0.35

Worm culture (earthworms) was placed in the prepared nutrient substrate, from consideration

30-100 thousand individuals of all ages at two square meters. The duration of complete processing of the nutrient substrate in a section, in this case was 6 months. During the experimental period the nutrient substrate was sprayed with water (as needed). Sectors for worm cultivation were covered with straw in order to reduce evaporation - summer and protection from cold - winter.

During the experimental period, were respected the requirements of the technology of bioconversion of organic waste by worm-culture (humidity - 70-80%, ammonia content 1.0-20 mg/kg, active acidity (pH) - 6.8-7.6 conventional units and cellulose content - 30%).

After 30 days from the beginning of the experiment, it was found that the worm-culture processed a substantial part of the nutrient substrate; therefore, the addition of the additional nutritive substrate was started. This process took place every 10-14 days. At the end of the experimental period, the worm-culture was separated from the substrate and placed in other sectors, prepared in advance for the development of the bioconversion technology of organic waste. As a result of the processing of organic waste, the valuable organic fertilizer –worm compost was obtained (Figure 1).



Figure 1. Worm compost - organic fertilizer obtained from cattle manure

Worm compost is one of the final products of the bioconversion of biodegradable waste obtained as a result of the vital activity of worm culture. It is made up of small dark brown granules, with the smell of soil after rain, is hygroscopic and can be stored in dry rooms for many years, without losing its qualities. The amount of obtained worm compost depends on

the type of biodegradable waste used as a nutrient substrate. From a ton of organic waste as a result of bioconversion, 700 kg of worm compost were obtained during six months. In Table 3 are shown the quality indicators of two fractions of worm compost obtained as a result of the bioconversion of organic waste by worm culture

Table 3. Quality indicators of worm-compost

Quality indicators	Worm compost fractions and indicator values, $M \pm m$	
	fraction 0.25 mm	fraction 1.00 mm
Active acidity (pH-ul), units	$7.81 \pm 0.03$	$8.08 \pm 0.02$
The organic substance, %	$24.39 \pm 0.45$	$27.41 \pm 0.41$
Total nitrogen, %	$1.09 \pm 0.01$	$3.00 \pm 0.04$
Potassium (K <sub>2</sub> O), %	$1.92 \pm 0.02$	$2.50 \pm 0.03$
Magnesium, %	$1.18 \pm 0.03$	$2.50 \pm 0.04$
Phosphorus (P <sub>2</sub> O <sub>5</sub> ), %	$1.37 \pm 0.08$	$2.50 \pm 0.06$
Calcium, %	$0.62 \pm 0.02$	$3.80 \pm 0.05$
Humus, %	$29.66 \pm 1.40$	$35.91 \pm 1.90$
Non-pathogenic bacterial flora, colonies	$2 \times 10^{12}$	$2 \times 10^{12}$

Comparing the values of the quality indicators of the worm compost in the two fractions, with those of the initial nutritive substrate, it was found that the active acidity, the content of total nitrogen, calcium, magnesium, potassium and phosphorus in the 0.25 mm fraction of the obtained worm compost, exceed that, from the nutrient substrate, respectively by 3.17%, 31.33%, 12.70%, 3.82 times, 2.11 times, and in the fraction of 1.00 mm, these indicators exceeded them, respectively by 6.74%, 3.61 times, 6.90 times, 2.14 times, 3.67 times and 3.84 times. The organic matter content decreased in the investigated fractions, respectively by 19.64% and 9.69%. As a result of the research, it was found that the worm compost contains 100 times more non-pathogenic microflora ( $2 \times 10^{12}$  colonies) than ordinary compost.

According to the obtained results, it was found that the worm compost is superior to the nutrient substrate. The organic substance during the bioconversion was transformed into humus. When incorporating worm compost into the soil, considerable savings are made, taking into account that 3-6 tons of worm compost are used per hectare compared to 40-70 t/ha of traditional compost.

According to the researches, it was found that one ton of worm compost contains 270-300 kg of humus. This allows to an essential reduce of the filling period of the amount of humus in the soil, thus restoring soil fertility and soil resistance to wind and alluvial erosion. Worm compost can be used to cultivate all species of agricultural plants, influencing beneficially on their physiological development and the yield obtained per unit area.

The period for obtaining worm compost from cattle manure lasted 8 months.

At the initial stage of the field experiment it was found that all crops in the experiment cultivated with worm-compost background, emerged 2 - 3 days earlier than those cultivated with mineral fertilizer and 5 - 7 days earlier than those in the control lots. This demonstrates that the worm compost beneficially influences the process of seed germination and emergence of agricultural crops.

Comparing the process of development of plants from all variants, it was found that in the lots with a background of worm compost, agricultural crops developed more intensely, the early flowering of alfalfa, the intense development of the rhizocarps in fodder beet and the formation of corn cobs took place with 5-6 days earlier than in the control lots and with 3-4 - earlier than in the groups with ammonium nitrate. Therefore, as a result of the performed studies, it was found that the incorporation of the worm compost in the soil, in a dose of 4 t/ha, led to the early physiological development of the crops, diminishing the flowering and ripening period of the cultivated plants.

To evaluate the quality of fodder crops, at the end of the experiment was determined the amount of nitrates in alfalfa, fodder beet and maize. The results of the investigations are set out in Table 4.

Analyzing the obtained results, it was found that in forage samples the amount of nitrates the nitrate content was varied, in some cases, exceeding the maximum permissible concentration (MPC), which for roughage is 500 mg/kg and for fodder beet - 800mg/kg. The amount of nitrates depended on half collection phase of vegetation and type of fertilizer used in the cultivation of forage crops.

Table 4. The content of nitrates in the forage crops

Types of forage crops	Variants of the experiment and the quantity of nitrates (mg/kg)		
	Control	Worm compost (4t / ha)	Ammonium nitrate (250 kg/ha)
Alfalfa Fin din lucernă	129.00 ± 1.10- 178.00 ± 0.97	200.50 ± 0.86- 207.00 ± 0.09	457.00 ± 1.74- 550.00 ± 1.15
Fodder beet	283.50 ± 0.66- 583.50 ± 6.19	376.00 ± 7.07- 631.00 ± 1.11	719.00 ± 2.11- 919.0 ± 5.31
Maize (stalks and the leaves)	157.80 ± 0.53- 257.80 ± 0.42	250.70 ± 0.46- 302.00 ± 0.81	926.4 ± 0. 46- 1113.00 ± 5.11

In all phases of vegetation of forage crops grown with fertilizer of ammonium nitrate was found a high content of nitrates. In samples of forage crops (in the last phase of vegetation), the amount of nitrates ascertained in alfalfa, fodder beet, and stems and leaves dry maize, it collected on lots with ammonium nitrate fund, surpassed that of control lot, respectively from 3.09 times, 1.58 times and 4.33 times.

At the end of the experiment, the concentration of nitrates in samples of alfalfa, fodder beet and maize, collected from the lots with ammonium nitrate exceeding the maximum permissible concentration (MPC), respectively by 175.00%, 14.88% and 39.13%

In the plants collected from the lots with worm compost, the nitrate content exceeded by 3.50% the value MPA, only in the alfalfa sample. In the samples fodder beet and the maize, the nitrate content was within the permitted limit (respectively 800mg/kg and 500 mg/kg).

In fodder collected on lot with fund of worm compost this indicator exceeds for 1.16-1.55 times (hay), 1.08-1.33 times (fodder beet) and 1.17-1.59 times (maize) that from plants of control lot, but did not exceed the maximum permissible concentration.

At the end of the experiment, an essential difference in forage crops harvest was found, depending on the background on which they were grown and the type of crop (Table 5).

Table 5. Influence of worm compost and mineral fertilizers on harvest of forage crops

Types of forage crops	Lots and quantity of obtained harvest				
	Control	With worm compost background (4 t/ha)		With ammonium nitrate background (250 kg/ha)	
		kg	kg	The increase production, %	kg
Alfalfa	650	1072	164.92	865	133.08
Fodder beet	690	1450	210.14	1173	170.00
Maize	420	693	165.00	567	135.00

It is necessary to mention that the harvest of alfalfa, fodder beet and corn obtained on the lots with worm-compost background was higher than on those with ammonium nitrate background. According to the results, the difference between the harvests of these two lots was 23.93% for alfalfa, 23.61% for fodder beet and 22.22% for maize.

Analyzing the results of the harvest of fodder crops, obtained from the experimental lots, it was found that the harvest of alfalfa, fodder beet and maize collected from the lots with worm compost background exceeded it by 64.92%, 110.14% and 65.00%, respectively, and the harvest collected from the lots with ammonium nitrate background exceeded, respectively by 33.08%, 70.00% and 35.00% that of the harvest of fodder crops from the control lots

Therefore, the worm compost influenced the early development of agricultural crops and the increase of the crop per unit area.

In the fodder crops cultivated on the lots with worm compost background, exceeding the content of nitrates was nonessential, remaining within the limits of the allowed value, and in those of the lots with ammonium nitrate background the concentration of nitrate was higher than the permissible.

Thus, the bioconversion of biodegradable organic waste through worm culture is a component part of organic agriculture and can be used in households with various forms of ownership.

## CONCLUSIONS

Bioconversion of organic waste through worm culture solves the important problems of organic agriculture: improving and reviving soil fertility; obtaining long-acting ecological organic fertilizers; increasing the harvest of agricultural crops; environmental protection.

The values of the qualitative indices of the worm compost essentially exceeded those of the nutrient substrate (partially fermented biodegradable waste).

The incorporation of worm compost in the soil, in the dose of 4 t/ha, influenced the early development of fodder crops, reducing the duration of the phenological phases.

In the crops cultivated with worm compost background, the content of nitrocomposites decreased, thus improving the quality of the obtained production.

The concentration of nitrates in samples of alfalfa, fodder beet and maize, collected from the lots with ammonium nitrate exceeding the maximum permissible concentration (MPC), respectively by 175.00%, 14.88% and 39.13%

Analyzing the results of the harvest of fodder crops, obtained from the experimental lots, it was found that the harvest of alfalfa, fodder beet and maize collected from the lots with worm compost background exceeded respectively, by 64.92%, 110.14% and 65.00%, and the harvest collected from the lots with ammonium nitrate background exceeded, respectively by 33.08%, 70.00% and 35.00% that of the harvest of fodder crops from the control lots.

Worm cultivation technology is an effective method for the development of organic agriculture.

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## ASSESSMENT OF SPRING BARLEY GENOTYPES RESISTANCE TO DROUGHT AT TWO DEVELOPMENTAL STAGES UNDER DIFFERENT SIMULATED CONDITIONS

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### Abstract

*Barley is a plant that can be grown in various conditions, from a pedoclimatic point of view, because it has a good drought tolerance. This study tested the drought tolerance of 25 spring barley genotypes using two drought induction methods, the polyethylene glycol method (PEG 10000) at NARDI Fundulea in a controlled climate (M1), and the sodium chlorate method (NaClO<sub>3</sub>) in the experimental field from ARDS Turda (M2). Drought stress is one of the most important issue for the two-row spring barley yield, since it simultaneously affects many properties at the morphological and physiological level, mainly the production elements and, implicitly, the final yield. Following this study, the Daciana and Jubileu varieties and spring barley perspective genotypes (4 lines from advanced generations To 2168-01, To 2115-10, To 2054-97 and To 2027-10) were shown to be tolerant to both types of induced drought (both in the seedling and adult plant developmental stages).*

**Key words:** drought tolerance, spring barley, polyethylene glycol, sodium chlorate.

### INTRODUCTION

Barley (*Hordeum vulgare* L.) is one of the oldest cultivated plants that has played a significant role in the development of human civilization, agronomic, physiological and genetic sciences in plant breeding. It was probably first used in human food in raw form or in the form of bread, porridge or soups. Barley will later be used mainly as fodder, in the manufacture of malt and beer and in the distillation processes (Boanta et al., 2019).

Barley is a plant that can be grown in various conditions, from a pedoclimatic point of view, because it has a good drought tolerance (Mishra & Shivakumar, 2000; Valenzuela & Smith, 2002 cited by Porumb, 2018) and is more resistant to high temperatures in the vegetation period than wheat, rye and oats (Muntean, 1993).

The demand and production of barley is growing for various reasons, namely: wide adaptability, the genetic diversity, being a model crop for molecular research and having a wide range of uses, such as food, in the beer

industry and animal feed. This is the reason why special importance is given to this plant through various studies at the molecular, cellular, physiological and morphological levels, barley having the ability to adapt more easily to the recent climate change (Kebede et al., 2019).

Drought is a major abiotic stress factor, which severely affects the crop production worldwide. The agronomic and physiological features associated with drought tolerance are adequate indicators in the breeding program (Rong et al., 2006) the identification of drought tolerant genotypes, in order to reduce the impact of water deficit on the crop yields.

Agricultural drought refers to a very low level of rainfall that cannot ensure the proper growth and development of crops during the growth season. Drought occurs for various reasons: quantitatively reduced rainfall, the timing of water availability depending on the requirements of each plant or the reduction of the soil water supply (Kebede et al., 2019).

Therefore, drought stress is an important aspect in the formation of barley production elements,

as it simultaneously affects many traits through morphological, physiological and metabolic changes that occur in all plant organs, eventually leading to a decreased production (Sacks et al., 1997; Cellier et al., 1998; Cochard et al., 2002 cited by Rong et al., 2006).

## MATERIALS AND METHODS

Within the theme of the ADER 2.1.2 research project, two studies were performed using two methods on drought tolerance of 25 genotypes of spring barley tested in two localities (Turda and Fundulea). At National Agricultural Research and Development Institute (NARDI) Fundulea, the spring barley genotypes were tested in the seedling phase by inducing stress with polyethylene glycol (PEG 10000), determining the root length, seedling height and leaf area. The seeds were sterilized with 1% sodium hypochlorite solution for 10 minutes, washed thoroughly with distilled water and germinated on rolls of filter paper in Berzelius beakers in tap water. They were kept in the growth chamber for five days at a temperature of 24°C and 16 hours of illumination. After five days, half of the rolls were further kept in tap water (control) and the remainder were transferred in to 15% polyethylene glycol solution (method M1). Both variants were kept in the growth chamber at the same parameters for two weeks (Petcu et al., 2020).

Using a ruler, biometric measurements were performed on the length of the stem, root and leaves (the length and width of the leaf were measured) and the leaf area was calculated using the following formula:

$$LA = (L * w * 0.66)$$

where:

LA - leaf area (mm<sup>2</sup>);

L - leaf length (mm);

w - leaf width (mm);

0.66 - the coefficient used to calculate the leaf area specific to barley.

At Agricultural Research and Development Station Turda (ARDS Turda), drought tolerance was tested according to the method of Blum et al., 1998 (method M2), by spraying the plants with sodium chlorate (NaClO<sub>3</sub>) 14 days after flowering. During the experiment, 4 rows with a length of 1 linear meter (two rows for

check and two for sodium chlorate treatment) were delimited for each experimental variant, where the treatment was applied by spraying this desiccant on the entire plant. Each variant was harvested individually and processed in the laboratory. Through this method, determinations were made on the yield elements, namely: ear length (EL), ear weight (EW), grains weight per ear (GW/E) and one thousand kernel weight (TKW). To determine these traits, 15 plants were analyzed in three repetitions.

The experiment was conceived as a two-factor type A × B, where: factor A is the treatment (with two graduations, check and treated); factor B is the genotype (with 25 graduations). The POLIFACT statistical analysis program was used to process the obtained data.

## RESULTS AND DISCUSSIONS

Assessing drought tolerance in the seedling stage is very important because it affects all subsequent stages and, ultimately, the yield per unit area. Such a study was also conducted by Sallam et al., in 2019. At this stage, the studies of genetic variation in drought tolerance were focused mainly on the characteristics of leaves and roots as in our case. Analysis of the variance revealed the significant effect of PEG-induced drought on the seedling height of spring barley genotypes (Table 1).

Table 1. Analysis of the variance for the seedling height at the spring barley genotypes studied

Source of variation	DF	MS	F
Factor A (Treatment)	1	106027.60	59.524*
Error A	2	1781.25	-
Factor B (Genotype)	24	1783.97	4.280***
Interaction (AxB)	24	963.09	2.310***
Error B	96	416.85	-

DF - Degrees of freedom; MS - Mean squares.

Along with the treatment factor (A), both genotypes (B) and the double interaction between treatment and genotype (A x B) have a more important contribution in the variance of seedling height, the values of sample F corresponding to the hereditary factor being very significant, suggesting differences between genotypes in terms of drought stress behavior. Similar results were obtained by Petcu et al. (2020) in a study conducted on 10 genotypes of winter barley.

The height of the seedlings in optimal humidity conditions was between 133 and 232 mm, and in drought conditions the recorded values were between 98 and 154 mm. The most affected variants were: the lines To 2172-01, To 2149-99, To 2013-99, To 2011-92, To 2198-13 and the Adina variety with the biggest differences being distinctly negative (Table 2).

Table 2. The effect of induced drought in seedling height in a series of spring barley genotypes tested at NARDI Fundulea

No.	Genotype	Seedling height (mm)			
		Check	Treatment (drought)	Diff.	Sign.
1	Daciana	174	140	-34	-
2	Turdeana	191	128	-53	-
3	Romanița	197	131	-66	0
4	<b>Adina</b>	<b>210</b>	<b>130</b>	<b>-80</b>	<b>00</b>
5	To 2270-94	195	145	-50	0
6	<b>To 2198-13</b>	<b>215</b>	<b>141</b>	<b>-74</b>	<b>00</b>
7	To 2096-10	187	151	-36	-
8	<b>To 2172-01</b>	<b>232</b>	<b>118</b>	<b>-114</b>	<b>00</b>
9	To 2168-01	187	125	-62	0
10	To 2115-94	173	123	-50	0
11	To 2036-02	189	136	-53	0
12	To 2054-97	195	138	-57	0
13	<b>To 2013-99</b>	<b>195</b>	<b>126</b>	<b>-70</b>	<b>00</b>
14	To 2095-01	208	154	-54	0
15	<b>To 2149-99</b>	<b>206</b>	<b>110</b>	<b>-96</b>	<b>00</b>
16	To 2017-93	174	127	-47	0
17	To 2014-99	207	157	-51	0
18	To 2247-01	150	126	-24	-
19	To 2167-01	133	126	-7	-
20	To 2051-10	188	131	-56	0
21	To 2123-01	197	145	-52	0
22	To 2027-10	123	111	-12	-
23	To 2170-01	161	98	-63	0
24	<b>To 2011-92</b>	<b>192</b>	<b>122</b>	<b>-70</b>	<b>00</b>
25	Jubileu	185	137	-49	0
LSD (p 5%)		41 mm			

Diff. - difference; Sign. - significance.

If in the case of seedling height (Table 1) the factor F registered significant values for the treatment as source of variation, in the case of root length, the analysis of variance showed the participation of both factors A (treatment) and B (genotype) and also their interaction in expressing this traits (Table 3).

Table 3. Analysis of the variance for root length of studied spring barley genotypes

Source of variation	DF	MS	F
Factor A (Treatment)	1	163152	194.099***
Error A	2	840.56	-
Factor B (Genotype)	24	1583.22	3.163***
Interaction (AxB)	24	1284.75	2.567***
Error B	96	500.50	-

DF - Degrees of freedom; MS - Mean squares.

It is obvious that the induced water stress affected the root system of the seedlings more than their aerial part. In terms of a well-developed root system in both environmental conditions, the genotypes Daciana, Turdeana, Romanița, Adina, To 2270-94 and To 2095-01 can be noticed.

On the other hand, the most affected genotypes under conditions of induced water stress were the varieties Turdeana, Romanița, Jubileu and the To 2036-02 line (Table 4). This is reflected in the differences between the check and the treatment as well as in their (very significant negative) meanings and it seems that these genotypes are not drought tolerant regarding the root system (Table 4). In this case it have to analyze them in relation to the length of the root under normal condition where these have the most developed root system. After exposure to induced drought condition, however, they have the most developed root system compared to other genotypes.

Table 4. The effect of induced drought on the root length in a series of spring barley genotypes tested at NARDI Fundulea

No.	Genotype	Root length (mm)			
		Check	Treatment (drought)	Diff.	Sign.
1	<b>Daciana</b>	<b>194</b>	<b>158</b>	-36	-
2	<b>Turdeana</b>	<b>249</b>	<b>128</b>	<b>-121</b>	<b>000</b>
3	<b>Romanița</b>	<b>251</b>	<b>146</b>	<b>-105</b>	<b>000</b>
4	<b>Adina</b>	<b>242</b>	<b>153</b>	<b>-89</b>	00
5	<b>To 2270-94</b>	<b>215</b>	<b>149</b>	<b>-66</b>	00
6	To 2198-13	232	141	-92	00
7	To 2096-10	204	131	-73	00
8	To 2172-01	181	147	-34	-
9	To 2168-01	209	135	-74	00
10	To 2115-94	180	121	-60	00
11	<b>To 2036-02</b>	<b>221</b>	<b>104</b>	<b>-117</b>	<b>000</b>
12	To 2054-97	221	153	-68	00
13	To 2013-99	195	143	-52	0
14	<b>To 2095-01</b>	<b>228</b>	<b>132</b>	<b>-96</b>	00
15	To 2149-99	208	123	-85	00
16	To 2017-93	205	155	-50	0
17	To 2014-99	192	137	-54	0
18	To 2247-01	133	132	-1	-
19	To 2167-01	196	142	-54	0
20	To 2051-10	186	150	-36	-
21	To 2123-01	179	149	-30	-
22	To 2027-10	194	137	-57	0
23	To 2170-01	159	104	-55	0
24	To 2011-92	189	144	-45	0
25	<b>Jubileu</b>	<b>221</b>	<b>121</b>	<b>-101</b>	<b>000</b>
LSD (p 5%)		40 mm			

Diff. - difference; Sign. - significance.

For the leaf area, the analysis of variance showed that the highest variation is due to

factor A (treatments) (Table 5). A similar situation is presented by Petcu et al. (2020) in a study conducted on 10 winter barley genotypes (varieties and lines).

Kang (1998) states that the genotype-environment interaction is a significant challenge in the genotype development. Table 5 shows the influence of the double interaction in expressing the variance of the leaf area. Water stress inhibits the growth of leaf area by disrupting photosynthesis and the metabolic processes due to stomatal closure, and tissue dehydration, respectively (Petcu et al., 2020).

Table 5. Analysis of the variance for the leaf area of the studied spring barley genotypes

Source of variation	DF	MS	F
Factor A (Treatment)	1	3408081	338.40***
Error A	2	10071.23	-
Factor B (Genotype)	24	20902.40	3.81***
Interaction (AxB)	24	15450.38	2.82***
Error B	96	5488.07	-

DF - Degrees of freedom; MS - Mean squares.

Among the studied traits, it seems that the leaf area is the most affected trait in conditions of induced water stress, fact highlighted in Table 6 from the very low values in treatment conditions compared to the check, as well as from the very significantly negative differences. The most affected genotypes in this respect were the spring barley varieties Turdeana, Romanița, Adina and the lines To 2172-01, To 2013-99, To 2149-99, To 2011-92.

The reduction in plant height may be due to the shortening of the internodes or the reduction of the leaf area (Jafarzadeh and Poostini, 2004). This connection between the leaf area and the height of the plants is reflected by the behavior of the above mentioned lines (To 2172-01, To 2149-99, To 2013-99 and To 2011-92), which were among the most affected by the water stress induced, both in the case of plant height and of the leaf area. Elements of yield that breeders use in assessing drought tolerance for barley and wheat include the seedling vigor, plant height, the number of days to maturity, ear length, the number of ear spikes, root length, the number of grain ears, grain weight, etc. (Sallam et al., 2019). Drought tolerance as a property can be assessed by any of these traits or by using drought indices that accurately assess the response of genotypes to drought stress.

Using the method proposed by Blum (1998), to test drought tolerance, we applied desiccant 14 days after anthesis, making determinations on ear length, ear weight, grains weight/ear and TKW. Observing the significance of the F factor for the treatments factor we can say that the variance of the length of the ear is not that much influenced by environmental conditions. An important contribution in expressing this feature seems to be the genetic factor (genotype), which records very significant values (Table 7).

Table 6. The effect of induced drought on the leaf area in a series of spring barley genotypes tested at NARDI Fundulea

No.	Genotype	Leaf area (mm <sup>2</sup> )			
		Check	Treatment (drought)	Diff.	Sign.
1	Daciana	510	215	-296	00
2	<b>Turdeana</b>	<b>552</b>	<b>172</b>	<b>-380</b>	<b>000</b>
3	<b>Romanița</b>	<b>563</b>	<b>179</b>	<b>-384</b>	<b>000</b>
4	<b>Adina</b>	<b>578</b>	<b>182</b>	<b>-394</b>	<b>000</b>
5	To 2270-94	540	208	-332	000
6	To 2198-13	661	220	-441	000
7	To 2096-10	532	235	-297	000
8	<b>To 2172-01</b>	<b>603</b>	<b>129</b>	<b>-474</b>	000
9	To 2168-01	507	230	-277	00
10	To 2115-94	403	164	-240	00
11	To 2036-02	565	176	-389	000
12	To 2054-97	587	205	-381	000
13	<b>To 2013-99</b>	<b>548</b>	<b>148</b>	<b>-400</b>	<b>000</b>
14	To 2095-01	667	325	-309	00
15	<b>To 2149-99</b>	<b>662</b>	<b>166</b>	<b>-463</b>	<b>000</b>
16	To 2017-93	540	310	-229	00
17	To 2014-99	603	355	-247	00
18	To 2247-01	383	227	-156	0
19	To 2167-01	287	197	-90	-
20	To 2051-10	525	311	-214	00
21	To 2123-01	472	245	-227	00
22	To 2027-10	342	197	-145	0
23	To 2170-01	411	162	-249	00
24	<b>To 2011-92</b>	<b>543</b>	<b>240</b>	<b>-302</b>	<b>00</b>
25	Jubileu	515	293	-222	00
LSD (p 5%)		132 mm <sup>2</sup>			

Diff. - difference; Sign. - significance.

Table 7. Analysis of variance for ear length (cm) in spring barley genotypes tested at ARDS Turda

Source of variation	DF	MS	F
Factor A (Treatment)	1	6.78407	59.579*
Error A	2	0.11387	-
Factor B (Genotype)	24	1.13417	2.709***
Interaction (AxB)	24	0.29684	0.709
Error B	96	0.41871	-

DF - Degrees of freedom; MS - Mean square.

The length of the ear for the plants tested in normal humidity conditions was on average

9.4 cm, with the highest values recorded by the genotypes Turdeana, Romanita, lines To 2096-10, To 2115-94, To 2017-93 and To 2247-01.

Under induced drought condition by the application of desiccant, average values of 9.0 cm were recorded, the most affected being the lines To 2149-99 and To 2115-94.

The least affected by the drought were the varieties Daciana, Jubileu and To 2198-13, To 2168-01, To 2167-01 and To 2011-99 lines (Figure 1).

Another important quantitative trait is the weight of the ear and the grains weight/ear. The

genetic variation in grain size and seed germination rate allows for a high flexibility of plants in response to genotypes in varying environmental conditions (Giles, 1990, quoted by Ellis & Marshal, 1998). Variability is the basic phenomenon for plant improvement and consists of the appearance of different individuals at the genetic level, differences that are also due to the interaction of the genotype with the environmental conditions (Jalata et al., 2011).

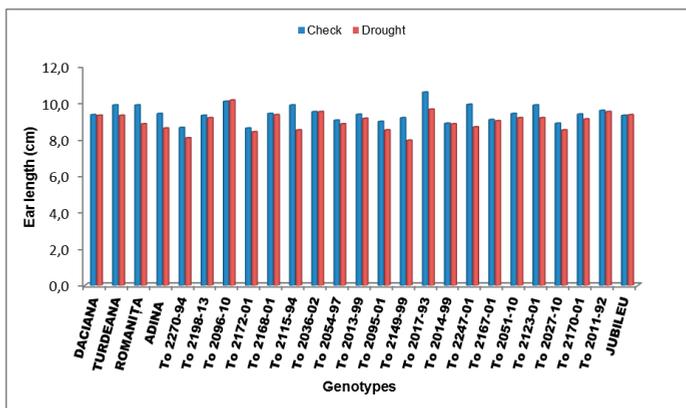


Figure 1. The effect of induced drought on ear length in spring barley genotypes, ARDS Turda

The analysis of the variances for ear weight and grain weight/ear is presented in Table 8. The treatment factor has a very significant involvement in the variability of the two traits (EW and GW/E), so the effects of induced drought and extreme heat in the post anthesis period can be observed. Even in the conditions of chemical desiccation, the values of the

variance corresponding to the treatment for the two properties ( $s^2 = 2.39$  and  $s^2 = 1.80$ ) suggest that the environment has the most significant contribution in their variation. Also, the genetic factor (genotype) is of particular importance in expressing these traits, presented very significant values (Table 8).

Table 8. Analysis of variance for ear weight (g) and grains weight/ear (g) in spring barley genotypes tested at Turda

Source of variation	DF	EW (g)		GW/E (g)	
		MS	F	MS	F
Factor A (Treatment)	1	2.39402	156.978***	1.80841	379.438***
Error A	2	0.01525	-	0.00477	
Factor B (Genotype)	24	0.15275	3.019***	0.12697	4.075***
Interaction (AxB)	24	0.06485	1.282	0.06354	2.039***
Error B	96	0.05060	-	0.03116	

DF - Degrees of freedom; MS - Mean square.

Drought can occur during flowering and can extend until the grain filling, thus affecting the number of grains in the ear and the grains weight, two important components of yield.

Because yield is a complex polygenic-controlled trait, breeders often use indirect selection and utilize traits well correlated with this trait to improve yield potential under

normal environmental conditions (Sallam et al., 2014).

The average weight of the ear under normal environmental conditions is between 1.36 and 1.88 g, while under stress conditions caused by drought induced during the post anthesis spring barley period, this trait decrease significantly, registering values between 1.65 and 0.75 g (Figure 2).

The average grains weight/ear under normal conditions is between 1.09 and 1.54 g and in the conditions of chemical desiccation it decreases very significantly registering oscillating values, between 0.93-1.32 g/ear, the amplitude of variation between the maximum value under normal conditions and the minimum under stress conditions being 8.98 g (Figure 3).

The most affected genotypes from the application of the desiccant for both properties were the Turdeana variety, but also the To 2172-01 and To 2017-93 lines for both traits. The least affected genotypes by drought were the Daciana and Jubileu varieties, but also the lines To 2168-01, To 2115-10, To 2054-97 and To 2027-10, these being considered tolerant genotypes (Figure 2 and 3).

These statements are also supported by Sallam in a study conducted in 2019, saying that when wheat and barley plants are exposed to drought or heat stress during grain filling, photosynthesis decreases rapidly which reduces the assimilates available in the grain. Consequently, there is a dramatic reduction in grain weight (Sallam et al., 2019).

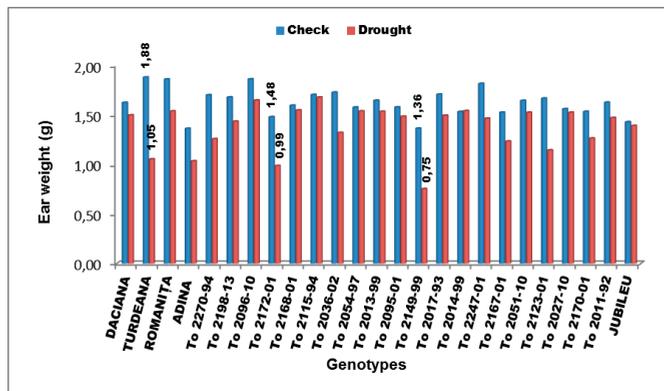


Figure 2. The effect of induced drought on ear weight (g) in spring barley genotypes tested in Turda

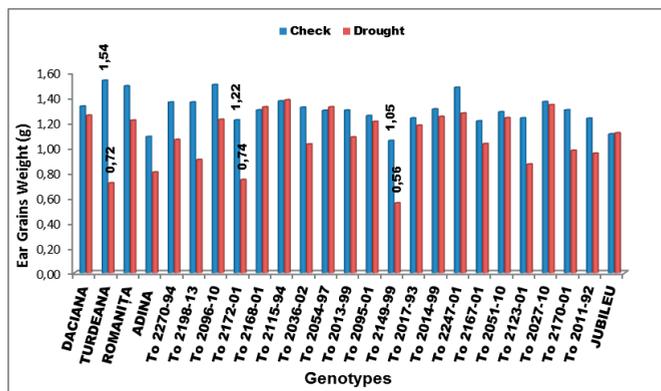


Figure 3. The effect of induced drought on grains weight/ear (g) in spring barley genotypes tested at Turda

Many papers in the speciality literature mention that, among the elements of yield, TKW is the least affected by environmental conditions

(Porumb et al., 2018), which can also be observed in our case, which implies that for TKW there are no meanings for factor F

(Table 9). However, looking at the values of the variance corresponding to the treatment for this property ( $s^2 = 144367$ ), it can be suggested that the environment in this case had a rather important contribution.

Table 9. Analysis of variance for TKW (g) in spring barley genotypes tested in Turda

Source of variation	DF	MS	F
Factor A (Treatment)	1	144367	1.334 <sup>ns</sup>
Error A	2	108252.40	-
Factor B (Genotype)	24	104966	0.976
Interaction (AxB)	24	108990.30	1.014
Error B	96	107515.30	-

DF - Degrees of freedom; MS - Mean square.

An important element of yield with indisputable implications on quality is TKW. The size of the embryo and implicitly the amount of reserve substances accumulated in the grain, necessary to ensure a good germination and at the same time a higher germination energy (Porumb, 2018) is closely related to this feature.

By directly reflecting the weight of the grains and indirectly their size, the genotypes analyzed in the present study under normal environmental conditions could be divided according to TKW values: medium grain genotypes, with TKW between 43-50 g and large grain genotypes, with TKW over 51 g. The most important values of this property in normal drought conditions were recorded by the Romanița variety (53.74 g). TKW values under stress conditions (Figure 4) ranged from 25.97 g (spring barley line To 2149-99) to 48.57 g (Daciana variety).

The wide range of values obtained from the application of the desiccant suggests the different impact of climatic conditions on this property and especially from the period from flowering to the time of treatment.

In addition to the Turdeana variety and the To 2172-01 line, which were noted and presented previously as the most affected, the To 2198-13 and To 2149-99 lines are also noted for TKW.

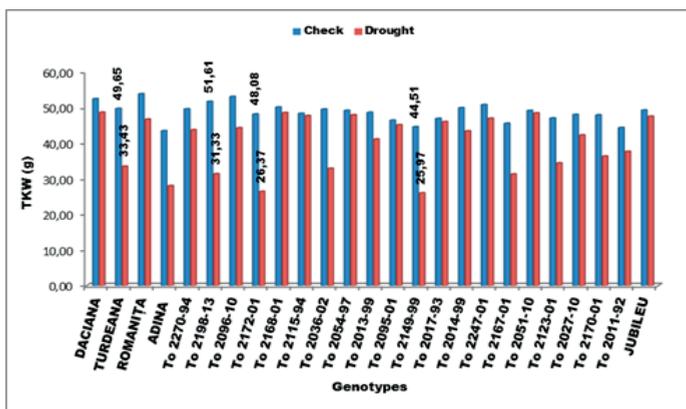


Figure 4. The effect of induced drought on TKW (g) in spring barley genotypes tested at Turda

## CONCLUSIONS

In terms of a well-developed root system under both normal and stress conditions, the spring varieties Daciana, Turdeana, Romanița, Adina, To 2270-94 and To 2095-01 lines can be noticed.

The lines To 2172-01, To 2149-99, To 2013-99 and the barley varieties Adina and Turdeana can be considered as very sensitive to both water stress and atmospheric stress, since they are most affected in both drought induced conditions (M1 and M2).

The genotypes affected to an insignificant extent by the drought induced by both methods (M1-PEG and M2-NACIO<sub>3</sub>) are varieties Daciana, Romanița, Jubileu and To 2198-13, To 2168-01, To 2011-92 and To 2095-01 lines. These can be considered drought tolerant as they do not show significant decreases in the values of the studied traits under normal conditions and in conditions of induced drought.

The Daciana variety stands out is distinguished by the most important values in conditions of

water stress or heat for most of the analyzed traits.

## ACKNOWLEDGEMENTS

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## ANTIFUNGAL ACTION OF ORGANIC APLICABLE FERTILIZERS TOWARDS PLANT PATHOGENS

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### Abstract

An antifungal action of novel organic agriculture applicable fertilizers manufactured on base of mixture of rock meal, produced from natural Austrian minerals was established in conducted *in vitro* trials towards economic important for the region of Bulgaria plant pathogens as *Monilia fructigena*, *Alternaria solani* and *Phytophthora infestans*. The tests reveal that even though these products are fertilizers, they can effectively act as protective fungicides. Additionally real field tests for phytotoxicity were conducted with different cultural and decorative plants and results show that there is absolutely no toxic action of these products even is 10 folds higher doses than labels recommended.

**Key words:** organic fertilizers, antifungal, plant pathogens.

### INTRODUCTION

Panamin fertilizers for organic agriculture are mixtures of rock meal, produced from natural Austrian minerals, with major components calcium, magnesium, silicon plus additional microelements like iron, phosphates and potassium (Panamin Co, 2017).

During recent years, this fertilizers became popular as in the commercial as in the organic agriculture. However it was founded that this products can express and ISR (Induced System Resistance) effect on plant i.e. can significantly reduce the attacks on treated plants caused by fungus and bacteria pathogens and provide resistance to frost and drought (Valchev and Valcheva, 2019; Kovacevik and Mitrev, 2019). In this study, conducted *in vitro* tests reveal that they can have also direct antifungal action towards some of the most economical important for the region of Bulgaria plant pathogens as *Monilia fructigena*, *Alternaria solani* and *Phytophthora infestans*.

The fact that such fertilizers can manifest ISR properties actually is not something unusual for the novel present day's fertilizer products.

Also even though ISR elicitors (promoters) usually do not have direct pesticidal properties, some of them may manifest such, especially in the higher concentrations (doses) (Reuveni and Reuveni, 1998).

### MATERIALS AND METHODS

Cultures of *Monilia fructigena*, *Alternaria solani* and *Phytophthora infestans* were isolated from infected fruits quince tree (for *Monilia fructigena*), tomato leaves (for *Alternaria solani*) and potato leaves (for *Phytophthora infestans*). Germ tube inhibition tests were conducted for determination of possible protective activity of the tested fertilizers. Fresh infected with inspected pathogen plant parts were collected and were incubated in a humid chamber for the purpose of stimulation the conidial sporulation of the phytopathogens. Conidial suspensions were prepared with the density  $3 \times 10^4$  spores/ml. Microscopic slides kind "handing drop" were sprayed with tested solutions and after drying, 20  $\mu$ l of conidial suspension was applied. The slides were incubated in a humid chamber, in thermostat and after 24-48 h. the number of germinated conidia was counted with a light microscope (Nikolov and Ganchev, 2011).

The possibility of evaluated fertilizers to inhibit the development of the mycelium of the tested phytopathogens was determined according to Thornberry methods (radial growth assays). For this purpose a potato dextrose agar (PDA) was used. The observation of mycelium growth was conducted on the 3rd, 7th, and 14th day (Secor and Rivera, 2012). The effectiveness was determined with formula of Abbott (Abbott,

1925). Statistical manipulation of the results was made with R Program Language for Statistical Computing (Team, 2013).

In this study five different products were evaluated: Panamin Agro, Panamin Suspension, Panamin ImunoActive, Panamin ImunoActive Plus and Panamin ImunoSafe

## RESULTS AND DISCUSSIONS

Trials with conidia spores reveal that products: Panamin ImunoActive and Panamin ImunoActive Plus can completely inhibit the germination of conidia spores of the all tested plant pathogens completely at 1 % (v/v) concentration. The rest of the products do not manifest such activity.

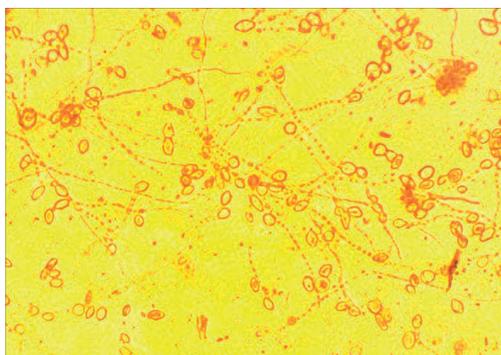


Figure 1. Conidial suspension of *Monilia fructigena* – control variant

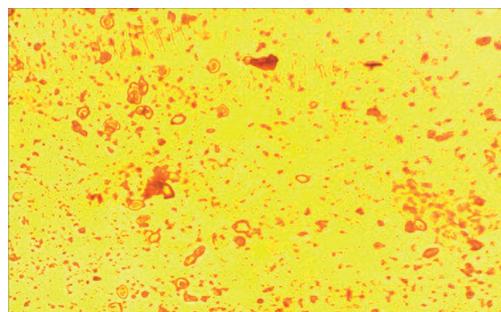


Figure 2. Conidial suspension of *Monilia fructigena* – Panamin ImunoActive – 1 % (v/v)

The conducted *in vitro* radial growth assays show also that both products: Panamin ImunoActive and Panamin ImunoActive Plus can express antifungal action towards tested plant pathogens at different degree. Panamin ImunoSafe at registered 0.3 % (v/v)

concentration do not show any pesticidal activity but in the 1 % and 3 % (v/v) was able to suppress the development of mycelium of testes pathogens.

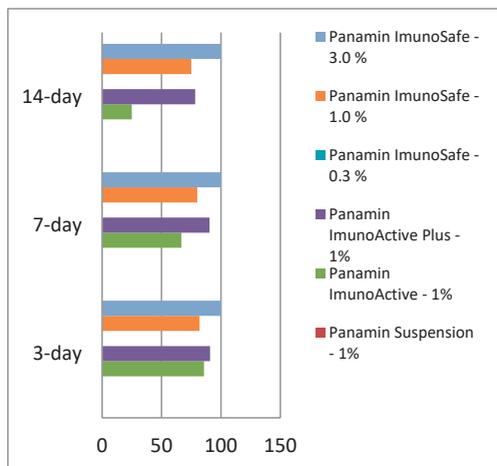


Figure 3. *Alternaria solani* radial growth assays

The figure above clearly show the ability of Panamin ImunoSafe completely to block the development of the mycelium at 3 % (v/v) concentration. The same product at 1 % (v/v) has a little bit less effectiveness while at registered 0.3 % (v/v) – zero effectiveness. Panamin ImunoActive has good level effectiveness only in 3 days after treatment, after that the effectiveness drop significantly. Panamin ImunoActivePlus however can provide satisfactory level of protection towards tested pathogen.

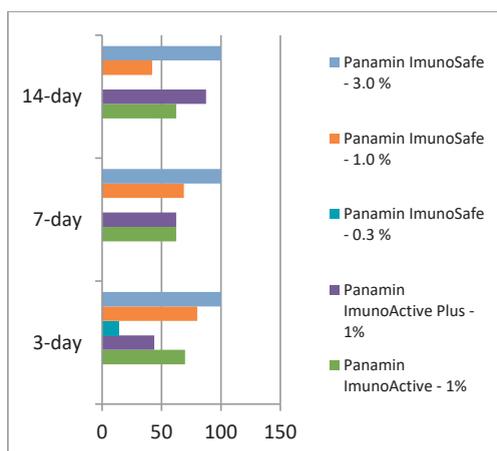


Figure 4. *Phytophthora infestans* radial growth assays

The results show that effectiveness of Panamin fertilizers towards *Phytophthora infestans* is significantly less than *Alternaria solani*. However Panamin ImunoSafe again at 3 % (v/v) concentration was able completely to inhibit the development of mycelium. The same product at 1 % (v/v) concentration show good effectiveness only 3 days after start of the test, after that the effectiveness drop significantly. Panamin ImunoActive also was not able to provide satisfactory level of effectiveness while Panamin ImunoActive Plus achieved approximately 87 % effectiveness 14 days after start of the test.

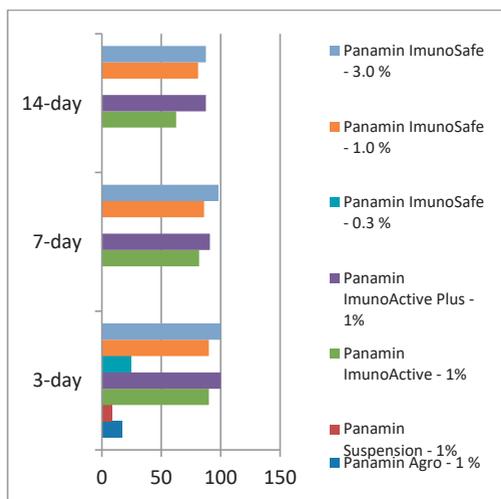


Figure 5. *Monilia fructigena* radial growth assays

According to the given tested plant pathogen, Panamin ImunoSafe together with Panamin ImunoActive Plus has higher level of effectiveness – over 87 % at the end of the test. Panamin ImunoActive have high effectiveness at the beginning of test but it drop to the 60 % at the end. Panamin ImunoSafe at 1 % (v/v) concentration also achieves good level of effectiveness. Surprisingly Panamin Agro,

Panamin Suspension and Panamin ImunoSafe at 0.3 % (v/v) concentration also express (although very low) effectiveness towards *Monilia fructigena* at the beginning of the test.

## CONCLUSIONS

The conducted trials reveal that some of Panamin fertilizers can express a excellent level of effectiveness towards some economic important plant pathogens. This is additional option for use of such kind products with can simulate the growth of the plants and can provide plant protection action simultaneously. The conducted phytotoxicity tests reveal that even in 10 folds higher doses than labels recommended, these products do not manifest any symptoms of phytotoxicity on plants.

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## CHANGES IN PRODUCTIVITY INDICATORS OF AGROCENOSIS IN *MISCANTHUS GIANTUS* WITH DIFFERENT METHODS OF WEEDS CONTROL

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### Abstract

*Deforestation without their subsequent restoration leads to the disruption of natural ecosystems. This led to the need to create fast-growing artificial plantations. From this point of view, heightened interest is shown in the plant of the bluegrass family - the giant miscanthus. As a renewable source of raw materials, it can become an alternative to forest crops. Differing in slow growth in the initial period, miscanthus weakly competes with weeds. Therefore, the purpose of the research was to determine the optimal way to combat weeds, ensuring the creation of a highly productive agrocenosis of Miscanthus giant in the forest-steppe of the Middle Volga region. In this regard, in 2015-2017, on the light gray soil of the experimental plot of the Penza State Agrarian University, a field experiment was laid on agrotechnical and chemical measures to control weeds in the culture of miscanthus. On average, over three years, the use of herbicides Magnum and Ballerina against the background of Tornado improved the conditions for the formation of leaves and before leaving for winter their area was the largest 18.36 and 19.09 thousand m<sup>2</sup>/ha, respectively, FP 496.1 ... 508.7 thousand .m<sup>2</sup> · day/ha, NPF decreased to 0.01 ... 0.08 g/m<sup>2</sup> · day. due to shading and death of the lower leaves. The maximum yield of dry matter of 2.96 t/ha was obtained with a double herbicide treatment. The main component that forms the frame of a plant is cellulose, the content of which in the aboveground mass was 56.96 ... 60.57%. The mass fraction of crude ash did not exceed 4.59%, potassium and calcium in it was 0.54% and 0.2%, respectively, the amount of nitrogen was 0.87%.*

**Key words:** *Miscanthus giganteus*, introduction, productivity.

### INTRODUCTION

The reason for the inevitable transition of society to resource-saving technological processes is the intensive and irrational use of the primary natural resource, which leads to the depletion of natural resources, especially the exhaustible sources of raw materials, which account for 81% of the final world energy consumption. Renewable energy sources account for 18%, which also includes biomass, which includes specially grown plants for energy, including trees. (Beringer et al., 2011; Khakhulaet et al., 2020)

However, deforestation without their subsequent restoration leads to disruption of natural ecological systems. Therefore, it became necessary to create artificial plantations, where plant growth is usually faster (Davis et al., 2013)

From this point of view, heightened interest is shown in the plant of the bluegrass family - the giant miscanthus (*Miscanthus giganteus*). As a

renewable source of raw materials, it can become an alternative to forest crops, which will take at least 80-100 years to recover. (Gushina et al., 2020) A fast-growing perennial herb is capable of forming colossal potential productivity - up to 40 t / ha of dry matter, since it belongs to the group of plants with the C4 photosynthesis type, which effectively uses solar radiation and water (Wang et al., 2011).

The average annual increase in the biomass of miscanthus is much higher than the average increase in wood in Russian forests, that is, aspen grows annually by 2.7 t/ha, birch - by 3.4, pine - by 3.6 t/ha. Miscanthus gives an annual increase of up to 9.3 t/ha and it is positioned as a promising cellulose-containing raw material (CCRM) for the isolation of cellulose and the production of products of its chemical modification, as well as biochemical transformation into glucose-pentose hydrolysates with subsequent conversion into ethanol, lactic acid, bacterial cellulose, etc.,

since its biomass is almost 60% cellulose (Makarova, 2013; Namsaraev et al., 2018). However, such productivity of miscanthus is not achieved in Russia. This is due to the slower growth of plants in the initial period, when rapidly developing weeds compete with it in the consumption of moisture, nutrients and light. Therefore, the purpose of the research was to determine the optimal way to combat weeds, ensuring the creation of a highly productive agrocenosis of *Miscanthus* giant in the conditions of the Middle Volga region.

## MATERIALS AND METHODS

In this regard, in 2015-2017. On the basis of the experimental site of the Penza State Agrarian University, a one-factor field experiment was carried out on light gray soil, characterized by the following agrochemical indicators in the arable horizon: humus content - 2.7% (GOST 26213-91), alkaline hydrolyzable nitrogen - 102.8 mg/kg (according to Cornfield), mobile phosphorus and exchangeable potassium - 188 and 110 mg/kg, respectively (GOST 26204-91), pH(KCL) - 5.7 (GOST-26483-75).

The experimental scheme includes agrotechnical and chemical measures to control the weed component in the agrocenosis of miscanthus. 1. Absolute control (control 1); 2. Production control (control 2 - two inter-row treatments); 3. Treatment with herbicide Tornado 500 (4 l/ha); 4. Treatment with herbicide Ballerina (0.6 l/ha); 5. Treatment with herbicide Magnum (0.01 kg/ha); 6. Treatment with herbicide Tornado 500 (4 l/ha) + treatment with herbicide Ballerina (0.6 l/ha); 7. Treatment with herbicide Tornado 500 (4 l/ha) + treatment with herbicide Magnum (0.01 kg/ha). The repetition was fourfold; the placement of the plots was systematic.

Soil cultivation consisted of autumn plowing to a depth of 20-25 cm with preliminary application of the herbicide Tornado 500 in mid-August, early spring harrowing and cultivation before planting miscanthus, which was carried out with rhizomes to a depth of 10 cm with a density of 20 thousand pcs/ha and a row spacing of 75 In the first and third years of the experiment, miscanthus was planted on May 6 and 4, respectively, in 2016 - April 16,

since there was an early and friendly spring with a temperature exceeding the norm by 4.5. Observations, records and analyzes were carried out according to generally accepted methods.

According to the moisture conditions, the first year of research was arid with a hydrothermal coefficient of 0.64, the second and third years were characterized by moderate (GTK-1.17) and sufficient (GTK-1.29) moisture.

## RESULTS AND DISCUSSIONS

The productivity of agrophytocenoses is determined by the amount of solar energy utilized in the process of photosynthesis, during which organic matter is formed, which determines the level of crop productivity. Controlling the photosynthetic activity of plants is one of the most effective ways to regulate their production processes that affect productivity (Weissmann et al., 2012).

As a perennial plant, miscanthus gigantic develops poorly in the first year of life. Therefore, a technique that accelerates the development of the assimilation surface of miscanthus can be a method of combating weeds in the year of its planting. On average, over three years by the harvesting period, which coincided with the end of September, the plants formed 5.19 ... 19.09 thousand m<sup>2</sup>/ha of photosynthetic surface. The autumn application of Tornado 500 increased the assimilation surface by 2.17 times, inter-row cultivation by 1.34 times.

The leaf area after plantation treatment with only systemic herbicides exceeded the absolute control by 1.96... 2.59 times. The use of Magnum and Ballerina on the Tornado background improved the conditions for the formation of the leaf apparatus and the area of miscanthus was the largest, 18.36 and 19.09 thousand m<sup>2</sup>/ha, respectively.

The total leaf area is characterized by the photosynthetic potential (PP), which is formed in accordance with the increase in the assimilation surface. By the end of the growing season, the highest rate was observed in plants grown under conditions of double herbicide treatment. On average, over three years, FP was 496.1 ... 508.7 thousand m<sup>2</sup> day/ha. In the absolute control, it was the lowest (136.8

thousand m<sup>2</sup> day/ha) due to the weak development of the photosynthetic surface, and with inter-row cultivation it increased by 1.37 times. The use of herbicides in their pure form increased the FP by 133.6 ... 215.4 thousand m<sup>2</sup> · day/ha. The indicator of the total dry biomass that is formed by plants during the day per square meter of "working" leaves is the net productivity of photosynthesis (NPF). On average, over the growing season, it was 0.32 ... 0.41 g/m<sup>2</sup> day. It is believed that the productivity of plants determines the accumulation of dry matter, which is a function of the assimilation process.

In dry 2015, miscanthus formed a rather low dry matter yield of 0.57 ... 2.18 t/ha (Table 1). However, its highest yield of 1.49 ... 2.18 t/ha was obtained with the use of herbicides Ballerina and Magnum based on the Tornado 500 background. Treatment of plantations with only systemic herbicides made it possible to improve the conditions for plant growth and increase the yield of dry matter, in comparison with the absolute control, by 0.33 ... 0.56 t/ha. With the introduction of a continuous herbicide in the fall, it increased by 0.83 t/ha, and with inter-row cultivation - by only 0.04 t/ha.

Table 1. Productivity of dry mass of *Miscanthus giant* first year of life, t/ha

Option	2015	2016 Nov.	2017	Average
Absolute control	0.57	1.33	1.19	1.03
Production control	0.61	2.21	1.60	1.47
Treatment with herbicide Tornado 500	1.40	3.24	1.76	2.13
Herbicide treatment Ballerina	1.13	3.68	1.80	2.20
Herbicide treatment Magnum	0.90	2.67	1.70	1.76
Herbicide treatment Tornado 500 + Ballerina	1.49	5.56	1.84	2.96
Herbicide treatment Tornado 500 + Magnum	2.18	4.57	2.15	2.97
Average by experience	1.18	3.32	1.72	2.07
NSR05	0.026	0.084	0.042	

The maximum dry mass yield of 1.33 ... 5.56 t/ha was obtained in 2016, which is 2.10 ... 3.73 times more than in the previous year and 1.12 ... 3.02 times more than in the next. In variants with double herbicide treatment, it was 4.57 ... 5.56 t/ha. The dry matter yield, in comparison with the absolute control, after the application of the herbicides Magnum and Balerina on the plantations was higher by 1.34 ... 2.35 t/ha, one herbicide Tornado 500 - by 1.91 t/ha. In the production control received 2.21 t/ha dry weight.

The biomass yield in 2017 varied within 1.19 ... 2.15 t/ha and the highest was obtained when using the systemic herbicide Magnum against the background of Tornado 500. Separate application of the continuous herbicide contributed to an increase in yield to 1.76 t/ha, and in combination with the herbicide Ballerina - up to 1.84 t/ha. In absolute control, 1.19 t/ha dry weight was obtained. The yield was slightly higher during inter-row cultivation of plantations (1.60 t/ha), and where the systemic

herbicides Magnum and Ballerina were used, the weight increased by 1.43 ... 1.51 times.

On average, over three years, the use of the herbicide Tornado 500, both separately and in combination with systemic herbicides, contributed to an increase in the yield of dry aboveground mass by 1.10 ... 1.94 t/ha. The lowest dry matter yield of 1.03 t/ha was obtained in absolute control. The year 2016 was optimal for the growth and development of *Miscanthus*. According to the results of three-year studies, on average, according to the experience, miscanthus against a natural background of fertility is able to form the yield of dry matter - 2.07 t/ha. The most productive are plantations where there was a complex application of herbicides of systemic action Ballerina and Magnum with Tornado 500.

The above analysis made it possible to reveal the patterns of changes in the yield of dry matter from weather conditions and methods of weed control. The maximum productivity potential of *Miscanthus giganteus* is manifested with an optimal combination of these factors

affecting an increase in the number of stems and their height.

For the production of cellulose in order to save the forest fund, non-woody plants are considered as promising sources of raw materials. Energy crops are also used to obtain solid (wood chips, pellets, briquettes), liquid and gaseous fuels.

The ash content shows how much ballast is in the fuel, which does not burn, and therefore does not bring any benefit. Typically, the ash content of solid fuels ranges from 0.5% (quality wood) to 50% (rice husks and pelleted droppings). Compared to wood, miscanthus is characterized by a fairly high ash content, which is explained by the metabolism of rapid growth (accumulation of nutrients). The mass fraction of crude ash over the years of research in plants of the first year of life varied slightly from 4.55 to 4.59%. Its highest indicator was noted in 2015, and the lowest in 2016.

One of the main elements that form ash are potassium and calcium. Potassium occupies a special place among the macronutrients most important for plants. In plants, it is most concentrated in young, growing tissues characterized by a high level of metabolism: meristems, cambia, young leaves and shoots. Promotes the synthesis of proteins and sugars, the movement and accumulation of carbohydrates in the productive parts of plants, normalizes the process of photosynthesis, increases the osmotic pressure of cell sap, thereby increasing drought resistance and winter hardiness of crops. This element also contributes to an increase in the mechanical strength of fabrics. The share of potassium in the dry matter of the crop in 2015 and 2017 was the same - 0.54%, in 2016 its content was 0.53%.

Calcium accumulates in adult organs, especially in leaves, serves as a neutralizer of oxalic acid harmful to plants and protects them from the toxic effects of various salts. Participates in the construction of cell membranes. The highest calcium content of 0.20% was found in plants of plantations in 2015, in subsequent years it was less by 0.01 ... 0.02%.

Nitrogen is a part of many organic compounds, the most important of which are amino acids, proteins, nucleic acids, chlorophyll, in addition, nitrogen contains phosphatides, vitamins, ATP,

alkaloids, etc. Young organs contain more nitrogen than old ones, and leaves more than stems. Energetic crops has a higher nitrogen content than wood. This can lead to high emissions of NO<sub>x</sub> during combustion, which is the collective name for oxides NO and NO<sub>2</sub>. But the combustion indicators of miscanthus chips are completely within the Austrian quality standard for fuel briquettes and pellets (in the Russian Federation there are no relevant standards yet). The dry mass over the years of research contained almost the same amount of nitrogen 0.86 ... 0.87%.

The main component that forms the carcass of a plant is cellulose. Cellulose with obsession in the aboveground mass by years of research was from 56.96 to 60.57%. The largest mass fraction of fiber was noted in 2016, the smallest in 2015, and in 2017 its content in dry matter did not exceed 58.76%.

## CONCLUSIONS

Thus, over three years of research, it was established that in 2016 the content of cellulose in the dry mass of miscanthus was the highest, and the ash and its components - the lowest, which is associated with favorable development conditions, which made it possible to obtain a more powerful aboveground biomass with the best quality indicators.

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## EVALUATION OF INCREASED RATES OF STARANE GOLD AT THE MAIZE HYBRID “BLASON DUO”

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### Abstract

In 2020 a field trial with the maize hybrid “Blason Duo” was conducted. The trial was stated on the experimental field of the Agricultural University of Plovdiv, Bulgaria. The performance of the herbicide product Starane Gold (100 g/l fluroxypyr + 1 g/l florasulam) in increased rates (1.20, 1.80 and 2.40 l ha<sup>-1</sup>) was evaluated. Untreated plot was accepted as a control. The efficacy and the selectivity of the herbicide, several biometrical parameters, and as well as the yields and its components were studied. The highest biological efficacy against the existing broadleaf weeds at the rates of 1.80 and 2.40 l ha<sup>-1</sup> were reported but phytotoxic symptoms score 4 (by the 9-score scale of European Weed Research Society) for the rate of 2.40 l ha<sup>-1</sup> were reported. Simultaneously with the decrease of the density of the broadleaf weeds a significant increase in the values of the studied traits as stem height and ear number per plant were found. The absolute and hectoliter seed mass as well as the yields were the highest for the treatment with application rates of 1.20 and 1.80 l ha<sup>-1</sup>.

**Key words:** *Triticum aestivum* L., efficacy, biometry, herbicide, yield.

### INTRODUCTION

Maize (*Zea mays* L.) is main grain and forage crop with adaptability to different agro-ecological conditions. That is the reason for the successful growing of the crop in many regions around the world. In Bulgaria it is strategical field crop. Maize has the highest energy value in comparison to the others forage crops (Tomov and Yordanov, 1984).

One of the main negative factors for agricultural production is the weeds. They decrease the yields and the quality of corn grain (Mitkov et al., 2019; Changsaluk et al., 2007; Tonev et al., 2007; Werner et al., 2004; Tonev, 2000; Masqood et al., 1999; Vengris et al., 1955). Weeds are annually emerging all over the fields and are causing great damage of the maize production (Tonev et al., 2011).

The chemical weed control is the most distributed among farmers. It is effective, fast and easy to apply. The proper herbicide application reduces the weed management costs up to 60% (Valcheva, 2011).

Today, high-yield agriculture is dependent on herbicides as they are an important component of weed management practices (Goranovska and Yanev, 2016). Nowadays weed control

with post emergence herbicide application at maize is rising (Whaley et al., 2006; Airoldi, 2000). In maize, chemical weed control is mainly performed by application of broad-leave and soil herbicides.

The aim of our study is to evaluate the efficacy and selectivity of Starane Gold in maize.

### MATERIALS AND METHODS

The experiment was situated in the experimental field of the base for training and implementation of the Agricultural University of Plovdiv, Bulgaria. The trial was conducted by the randomized block design in 3 replications. The size of the experimental plot was 10 m<sup>2</sup>.

Variants of the trial were: 1. Untreated control; 2. Starane Gold (100 g/l fluroxypyr + 1 g/l florasulam) – 1.20 l ha<sup>-1</sup>; 3. Starane Gold - 1.80 l ha<sup>-1</sup>; 4. Starane Gold - 2.40 l ha<sup>-1</sup>.

The sowing was done in April, and the herbicide treatment is applied in 3rd - 5th leaf stage (BBCH 13-15). The trial was conducted with the maize hybrid “Blason Duo”. For removing the influence of the grass weeds the whole experimental area was treated with Stratos Ultra (100 g/l cycloxdim) at rate of 2.00

1 ha<sup>-1</sup>. The treatment with Stratos Ultra was done one week after the Starane Gold treatment.

Characteristics of the maize hybrid “Blason Duo”: FAO Group 450; tolerant to the herbicide cycloxydim; high-yielding hybrid forming ears low and uniformly (<https://euralis.bg//43-es-blason-duo/>)

Precrop of maize was the winter wheat variety “Enola”. On the whole experimental field combined fertilization with 250 kg ha<sup>-1</sup> with N:P:K (15:15:15), followed by deep ploughing was done. Before sowing of the crop, disking on the depth of 15 cm and two harrowings on 8 cm of depth as well as spring dressing with 250 kg ha<sup>-1</sup> NH<sub>4</sub>NO<sub>3</sub> was also performed.

The efficacy of the studied herbicide rates against the weeds by the 10 score scale of EWRS (European Weed Research Society) on the 14<sup>th</sup>, 28<sup>th</sup> and 56<sup>th</sup> day after application was studied. The selectivity of the herbicide by the 9 score scale of EWRS was also evaluated (Zheliashkov et al., 2017).

The natural broadleaf weed infestation was presented by the weeds *Xanthium strumarium* L.; *Chenopodium album* L.; *Amaranthus retroflexus* L.; *Solanum nigrum* L.; *Portulaca oleracea* L.

During the growing season, two of the studied traits were reported - stem height and number of ears. From each repetition at random (in the beginning, in the middle and at the end of the trial plot) 12 plant total were measured.

After harvest, measurements of three quantitative traits were done – ear length and diameter as well as number of seeds per ear. Ten maize ears from each replication were measured.

From each variant for the three repetitions, 1000 air-dried seeds were counted and the absolute mass of the grains in grams was established. The hectoliter mass in kg was calculated for each replication.

Due to the close values of the indicators between the different variants, the comparisons were performed only with the control and the other variants. The initial data were processed by analysis of variance and variation using SPSS 19 program.

The maize grain yield (kg ha<sup>-1</sup>) was recorded by weighting of the seeds of 10 maize ears for each treatment in three replications. The

obtained results were divided on 10 and the grain yield was recalculated multiplying the result by the number of plants in one hectare.

The data for the grain yield were processed by Duncan’s multiple range test (p<0.05).

## RESULTS AND DISCUSSIONS

The obtained results regarding the efficacy of Starane Gold against the present weed species on the 14<sup>th</sup>, 28<sup>th</sup> and 56<sup>th</sup> day after application is presented. The efficacy is lower in the beginning of the reporting period and it is increasing in time.

On Table 1 is presented the efficacy of Starane Gold against *Xa. strumarium*. This species is among the most dangerous weeds in maize (Karimmojeni et al., 2010). In a trial conducted by Konstantinović and Korać (2011) 86% efficacy against *Xa. strumarium* was reported after the evaluation of Starane 300. In this experiment, for all evaluated rates of the herbicide product, excellent efficacy against this noxious weed was recorded - 95-100%. Mitkov (2020) also reported 95% efficacy for the evaluated rates (1.20 and 1.50 l ha<sup>-1</sup>) of Startane Gold.

Table 1. Efficacy of Starane Gold against *Xa. strumarium*, %

Treatments/days after application	14 <sup>th</sup>	28 <sup>th</sup>	56 <sup>th</sup>
1. Untreated control	-	-	-
2. Starane Gold - 1.20 l ha <sup>-1</sup>	75	90	95
3. Starane Gold - 1.80 l ha <sup>-1</sup>	80	95	100
4. Starane Gold - 2.40 l ha <sup>-1</sup>	90	100	100

On table 2 is presented the efficacy of Starane Gold against the weed *Ch. album*.

Table 2. Efficacy of Starane Gold against *Ch. album*, %

Treatments/days after application	14 <sup>th</sup>	28 <sup>th</sup>	56 <sup>th</sup>
1. Untreated control	-	-	-
2. Starane Gold - 1.20 l ha <sup>-1</sup>	25	35	45
3. Starane Gold - 1.80 l ha <sup>-1</sup>	40	55	55
4. Starane Gold - 2.40 l ha <sup>-1</sup>	65	75	85

This species was the most difficult-to-control in the trial, independently the applied herbicide rate. Even the rate of Starane Gold - 2.40 l ha<sup>-1</sup> did not ensure 100% control. On the 56<sup>th</sup> day after treatment 85% efficacy was found. In our previous study we reported excellent (100%)

control of *Ch. album* after the application of Principal Plus WG - 0.44 kg ha<sup>-1</sup> + Trend 90 - 1.00 l ha<sup>-1</sup> (Mitkov et al., 2019).

The efficacy data against *A. retroflexus* is presented on Table 3. Damalas et al. (2018) report that *A. retroflexus* can be successfully controlled after application of with herbicide mixtures based on tembotrione + rimsulfuron, nicosulfuron or foramsulfuron. In our study it is found that the weed can be successfully controlled by all studied Starane Gold doses.

Table 3. Efficacy of Starane Gold against *A. retroflexus*, %

Treatments / days after application	14 <sup>th</sup>	28 <sup>th</sup>	56 <sup>th</sup>
1. Untreated control	-	-	-
2. Starane Gold - 1.20 l ha <sup>-1</sup>	75	85	95
3. Starane Gold - 1.80 l ha <sup>-1</sup>	80	95	100
4. Starane Gold - 2.40 l ha <sup>-1</sup>	95	100	100

All evaluated rates of the herbicide product were able to control the weed *S. nigrum* from the first evaluation date to the 56<sup>th</sup> after the treatments (Table 4). We have found excellent control of this species in our experiments conducted with different herbicide products in the past (Tonev et al., 2016; Mitkov et al., 2018; Mitkov et al., 2019).

Table 4. Efficacy of Starane Gold against *S. nigrum*, %

Treatments / days after application	14 <sup>th</sup>	28 <sup>th</sup>	56 <sup>th</sup>
1. Untreated control	-	-	-
2. Starane Gold - 1.20 l ha <sup>-1</sup>	80	95	100
3. Starane Gold - 1.80 l ha <sup>-1</sup>	95	100	100
4. Starane Gold - 2.40 l ha <sup>-1</sup>	95	100	100

On Table 5 are the obtained results for the efficacy of Starane Gold against *P. oleracea*.

Table 5. Efficacy of Starane Gold against *P. oleracea*, %

Treatments / days after application	14 <sup>th</sup>	28 <sup>th</sup>	56 <sup>th</sup>
1. Untreated control	-	-	-
2. Starane Gold - 1.20 l ha <sup>-1</sup>	85	85	90
3. Starane Gold - 1.80 l ha <sup>-1</sup>	90	100	100
4. Starane Gold - 2.40 l ha <sup>-1</sup>	100	100	100

The reported efficacy is close to those recorded for the weeds discussed above. On the 56<sup>th</sup> day after treatments the efficacy is excellent for all evaluated rates - 90-100%. Herbicides are xenobiotics - "foreign" substances for the

plants. When the tolerance of cultivated plants to the absorbed herbicide is not enough to destroy the crop, the result is herbicide stress leading to various structural and functional distortions (Vischetti et al., 2002). The visual phytotoxicity recorded is presented on Table 6. Visual phytotoxic symptoms were not observed when the herbicide was applied in the rate of 1.20 l ha<sup>-1</sup> and the phytotoxicity score by the scale of EWRS was 0.

For the application the Starane Gold rare of 1.80 l ha<sup>-1</sup> phytotoxicity score on the 7<sup>th</sup> day after the treatments was 1. The observed symptoms were yellowing between the veins without affecting the midrib. The symptoms were observed in the leaf sheath. The phytotoxicity signs disappear completely on the 14<sup>th</sup> day after herbicide application. Mitkov (2020) also reported that the application of Starane Gold SE - 1.5 l ha<sup>-1</sup> caused temporary phytotoxicity that was completely overcome.

Table 6. Visual phytotoxicity of Starane Gold for maize, score by EWRS

Treatments / days after application	7 <sup>th</sup> day after treatment	14 <sup>th</sup> day after treatment
1. Untreated control	-	-
2. Starane Gold - 1.20 l ha <sup>-1</sup>	0	0
3. Starane Gold - 1.80 l ha <sup>-1</sup>	1	0
4. Starane Gold - 2.40 l ha <sup>-1</sup>	4	1

Greater phytotoxicity signs for the high rate (2.40 l ha<sup>-1</sup>) of Starane Gold were recorded. On the 7<sup>th</sup> day after treatment the visual phytotoxicity score was 4. The symptoms were expressed in yellowing between the veins not only in the area of the sheath, but on the whole leaf blade. Also crimping on the blade base was recorded.

On the 14<sup>th</sup> day after the herbicide treatment the plants overcome the herbicide stresses in some extend and the phytotoxicity score decreased to score 1. Later in time the visual herbicide toxicity disappeared, but stunning and growth retardation was recorded for treatment 4 (Starane Gold - 2.40 l ha<sup>-1</sup>).

The data for the growth parameters and its statistical analyses are presented on Table 7. The meanings of t are as follows: 2,001 (p<0.05), 2,663 (p<0.01) and 3,466 (p<0.001). After performing these analyses significant differences were not recorded. The increasing

of Starane Gold rates did not influence the growth and development in one-way performance. Only the trait stem height for the three evaluated doses showed a significant difference in comparison to the untreated control at level of significance  $p < 0.001\%$ . For

the weed control in the trial all three rates of the herbicide helped the maize hybrid grown in the experiment to show its growing potential (this trait is also determined to group I – the most distant group according to the control.

Table 7. Data analyses for 7 quantitative parameters of maize (growth and productive traits)

Stem height (m)					
Treatment	Average	Difference	t e	Significance	Group
1	2.40	-	-		IV
2	2.70	0.30	6.88	+++	I
3	2.80	0.40	10.56	+++	I
4	2.66	0.26	6.45	+++	I
Number of ears per plant					
Treatment	Average	Difference	t e	Significance	Group
1	1.08	-	-		IV
2	1.44	0.36	3.75	+++	I
3	1.69	0.61	6.73	+++	I
4	1.22	0.56	1.64	ns	IV
Ear length (cm)					
Treatment	Average	Difference	t e	Significance	Group
1	17.80	-	-		IV
2	18.77	0.97	1.71	ns	IV
3	19.24	1.44	2.55	+	IV
4	19.09	1.29	2.22	+	IV
Number of seeds per ear					
Treatment	Average	Difference	t e	Significance	Group
1	541.06	-	-		IV
2	571.46	30.40	1.12	ns	IV
3	586.26	45.20	1.91	ns	IV
4	561.06	20.00	0.81	ns	IV
Ear diameter (cm)					
Treatment	Average	Difference	t e	Significance	Group
1	3,53	-	-		IV
2	3,74	0.21	2.54	+	III
3	3,84	0.31	3.11	++	II
4	3,72	0.19	1.84	ns	IV
Absolute seed mass (g)					
Treatment	Average	Difference	t e	Significance	Group
1	352.92	-	-		IV
2	383.21	30.29	4.73	++	II
3	379.53	26.61	4.52	+	III
4	333.57	19.35	3.11	-	V
Hectoliter seed mass (kg)					
Treatment	Average	Difference	t e	Significance	Group
1	76.50	-	-		IV
2	77.10	0.60	2.00	ns	IV
3	77.30	0.80	2.50	ns	IV
4	74.60	-1.90	3.47	-	V

For the number of ears per plant for the rates of 1.20 and 1.80 l ha<sup>-1</sup> a similar influence was found. The highest examined rate of Starane Gold (2.40 l ha<sup>-1</sup>) decreased the number of ear per plant and the differences in comparison to the untreated control were insignificant. The

two treatments were situated in the same group – IV. The effect of the application of the lowest evaluated rates of the herbicide was not significantly expressed for these two indicators. The increase of the herbicide rate led to significant changes of the number of seeds per

ear and hectoliter seed mass. The growth parameters of the maize hybrid were mostly influenced by the rates of the studied herbicide product while the productive indicators had insignificant change in the values in comparison to the control.

Maize grain yields are presented on table 8. Studies conducted from different authors show that, depending on the type and degree of weed infestation, the maize grain yield may decrease from 24% to 96,7% (Mitkov, 2020; Mitkov et al., 2019; Mitkov et al., 2018; Tonev et al., 2016; Najafi and Tollenaar, 2005; Oerke and Dehne, 2004; Khan et al., 2003; Zhalnov and Raikov, 1996).

Table 8. Maize grain yields, t ha<sup>-1</sup>

Treatments / days after application	Grain yields	% of yield increase
1. Untreated control	4.97 c	100%
2. Starane Gold – 1.20 l ha <sup>-1</sup>	7.98 a	+60%
3. Starane Gold – 1.80 l ha <sup>-1</sup>	7.84 a	+57%
4. Starane Gold – 2.40 l ha <sup>-1</sup>	6.70 b	+35%

Values with different letters are with proved difference according to Duncan's multiple range test ( $p < 0.05$ ).

The highest yields for the treatments with Starane Gold – 1.20 l ha<sup>-1</sup> (7.98 t ha<sup>-1</sup>) and Starane Gold – 1.80 l ha<sup>-1</sup> (7.84 t ha<sup>-1</sup>) were recorded. For these two variants the yield increase is 60 and 57% in comparison to the untreated control respectively. The herbicide rate of 2.40 l ha<sup>-1</sup> led to yield decrease. It is important to remember that the yield decrease from uncontrolled weeds is higher than what might appear from injury by registered herbicide treatments (Hartzler, 2013). That statement was confirmed in our study. The productivity for treatment 4 (Starane Gold - 2.40 l ha<sup>-1</sup>) was 6.70 t ha<sup>-1</sup> - 35% increase when compared to the yield of the untreated control - 4.97 t ha<sup>-1</sup>.

## CONCLUSIONS

All studied rates of Starane Gold showed excellent control of the weeds *Xanthium strumarium* L., *Amaranthus retroflexus* L., *Solanum nigrum* L., and *Portulaca oleracea* L. The weed *Chenopodium album* L. was the most difficult-to-control in the study. The rate of

Starane Gold - 2.40 l ha<sup>-1</sup> ensured 85% efficacy on the 56<sup>th</sup> day after treatment.

The application of the high rate of the herbicide showed phytotoxicity symptoms that lead to depressed development of the plants, especially for treatment 4.

The highest yields for the treatments with Starane Gold – 1.20 l ha<sup>-1</sup> and Starane Gold – 1.80 l ha<sup>-1</sup> was recorded, and the rate of 2.40 l ha<sup>-1</sup> lead to yield decrease.

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## CORRELATION AND PATH-ANALYSIS OF MORPHOLOGICAL TRAITS AND YIELD OF DRY TOBACCO IN COMPLEX RESISTANT TO VIRAL DISEASES VIRGINIA TOBACCO LINES

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### Abstract

*The aim of the study is to evaluate the impact of morphological traits on the yield of Virginia tobacco lines with consolidated complex resistance to economically important viral diseases, Tobacco mosaic virus and Potato virus Y. The effect of the change in the height of the plant, the number of leaves, the sizes of the sixth and twelfth leaves on the yield is studied. Correlation analysis, regression analysis and Path analysis were applied. The direct and indirect influence of the studied traits on the yields is determined. The calculated correlation coefficients show a very strong, positive correlation between the yield and the length of the sixth leaf (0.985 \*\*), the width of the sixth leaf (0.949 \*\*), the number of leaves (0.934 \*\*), the length (0.970 \*\*) and the width of the twelfth leaf (0.966 \*\*). In L 5, the length of the sixth leaf and the width of the twelfth leaf have a very strong direct effect on the yield. In L 6.3.3, the number of leaves and the length of the sixth leaf have the highest direct effect on the yield.*

**Key words:** tobacco, Virginia, stable lines, yield, correlations, regression analysis.

### INTRODUCTION

Tobacco is an industrial plant of great economic importance (Maleki et al., 2011) and is the main livelihood for part of the population of a significant number of countries in the world with diverse climates and soils (Dimanov, 2014; Bozukov, 2014). In the selection of this crop the creation of high-yielding and quality varieties resistant to biotic and abiotic stress is of particular importance. Studies of the relation between yield and other agronomic traits are essential for understanding the direction of expected changes during the selection process (Josm, 2005). Correlations can be used as criteria for selection effect by phenotype (Popova et al., 2013). The application of correlation and regression analyzes in tobacco studying is becoming more widespread in scientific research (Wang et al., 1997; Dospataliev et al., 2014; Ahmed and Mohammad, 2017; Georgieva and Kirkova, 2018). After correlation and Path analysis, a strong positive correlation was found between

the number of leaves and the length of the largest leaves with the yield of the plant Wenping et al., (2009). A study of the relations between the yield of tobacco and plant height, number of leaves, leaf length and width, stem circumference, etc. shows that the yield is positively significantly correlated with all biometric traits (Maleki et al., 2011).

Kashif Ali Shah et al. (2016) study the phenotypic and genotypic correlation between yield elements and qualitative characteristics in Virginia tobacco. They found that there were significant positive correlations between yield and leaf area, number of leaves, weight of green leaves and grade index, which should be used for further evaluation in tobacco growing programs.

The aim of this study was to identify the morphological features that affect the productivity of complex resistant to potviruses and tobamoviruses Virginia tobacco lines, as well as to analyze the direct and indirect links between them and tobacco yield.

## MATERIALS AND METHODS

Two lines were studied: L 5 and L 6.3.3, having complex resistance to economically important viral diseases, Tobacco mosaic virus with tobamovirus agents and Sipaniza with potyvirus agents (Yonchev, 2015). The experiment is set in four repetitions with the size of the harvest plot of 27 m<sup>2</sup> with a planting scheme of 110-40/45 cm according to the technology adopted for Virginia tobacco in Bulgaria. The biometric analysis was performed on a total of 80 plants per variant. Plant height, number of leaves, length and width of the sixth and twelfth leaves (cm), yield of dry tobacco (kg/da) were reported. The obtained data were processed with the statistical software product SPSS 24 through correlation, regression and Path-analysis (Cronck, 2012; Field, 2013; Weinberg and Abramowitz, 2016).

## RESULTS AND DISCUSSIONS

As a result of the applied correlation analysis on the basis of experimental data at line L 5, a very strong, positive relation between plant height, number of leaves and sizes of the sixth and twelfth leaves on the yield was proved (Table 1). The calculated correlation coefficients show a significant positive relation between yield and plant height (0.794\*\*), as well as a very strong, positive correlation with the number of leaves (0.934\*\*), the length of the sixth leaf (0.985\*\*), the width of the sixth

leaf (0.949\*\*), the length (0.970\*\*) and the width of the twelfth sheet (0.966\*\*).

Table 2 presents the results of the correlation analysis for line L 6.3.3. The yield is in a significant positive relation with the length of the sixth leaf (0.781\*\*), moderate relation with the width of the sixth leaf (0.591\*), as well as in a strong, positive relation with the length (0.914\*\*) and the width of the twelfth leaf (0.818\*\*).

The decomposed general correlation coefficients, respectively, between dry tobacco yield and morphological traits at line L 5 of direct and indirect coefficient are shown in Table 3. They show that the different traits affect the economic qualities specifically. The general conclusion that should be drawn from the attached analysis is that the yield on the complex stable line L 5 is most strongly influenced by the length of the sixth leaf (18.49%), followed by the length of the twelfth leaf (17.93%), the width of the twelfth leaf (17.78%), the width of the sixth leaf (17.16%), the number of leaves (16.61%) and to a lesser extent the height of the plant (12.01%).

The high correlation coefficient between plant height and yield is largely due to the strong indirect influence between them. The result is similar for the number of leaves, width of the sixth leaf, length of the twelfth leaf. The length of the sixth leaf and the width of the twelfth leaf have a very strong direct effect on the yield, which determines the high correlation coefficient. Here the indirect influence is very weak and is fully compensated by the direct one.

Table 1. Correlation coefficients for economic and biometric indicators of a stable line L 5

	Yield da (kg)	Height of the plant (cm)	Number of leaves	Length of 6th leaf (cm)	Width of 6th leaf (cm)	Length of 12th leaf (cm)	Width of 12th leaf (cm)
Yield of dry tobacco da (kg)	1	0.794**	0.934**	0.985**	0.949**	0.970**	0.966**
Height of the plant (cm)		1	0.899**	0.850**	0.633*	0.754**	0.758**
Number of leaves			1	0.970**	0.881**	0.949**	0.956**
Length of 6th leaf (cm)				1	0.940**	0.976**	0.971**
Width of 6th leaf (cm)					1	0.979**	0.973**
Length of 12th leaf (cm)						1	0.996**
Width of 12th leaf (cm)							1

\*, \*\* Correlation coefficient at level of proof, 0.01 and 0.05, respectively

Table 2. Correlation coefficients for economic and biometric indicators of a stable line L 6.3.3

	Yield	Height of the plant (cm)	Number of leaves	Length of 6th leaf (cm)	Width of 6th leaf (cm)	Length of 12th leaf (cm)	Width of 12th leaf (cm)
Yield	1	0.513	0.288	0.781*	0.591*	0.914**	0.818**
Height of the plant (cm)		1	0.941**	-0.004	-0.306	0.678*	0.774**
Number of leaves			1	-0.254	-0.531	0.461	0.613*
Length of 6th leaf (cm)				1	0.949**	0.680**	0.516
Length of 6th leaf (cm)					1	0.451	0.274
Length of 6th leaf (cm)						1	0.955**
Length of 6th leaf (cm)							1

\*, \*\* Correlation coefficient at level of proof, 0.01 and 0.05, respectively

Table 3. Direct and indirect influences of the studied traits on the yield from line L 5

Trait	Direct coefficient (Beta)	Indirect (Path) coefficient	Correlation coefficient (R)
Height ( $x_1$ )	-0.035	0.829	0.794
Number of leaves ( $x_2$ )	-0.695	1.629	0.934
Length of the 6th leaf ( $x_3$ )	1.481	-0.496	0.985
Width of the 6th leaf ( $x_4$ )	-0.274	1.223	0.949
Length of the 12th leaf ( $x_5$ )	-0.6	1.570	0.97
Width of the 12th leaf ( $x_6$ )	1.083	-0.117	0.966

The mathematical model, presenting in analytical form, the influence of the six biometric indicators on the economic qualities of line L5, is of the type:

$$y=28.967-0.035x_1-13.061x_2+9.54x_3-2.748x_4-3.352x_5+9.422x_6.$$

The compiled model is statistically significant at a significance level of  $\alpha \leq 0.05$ .

Table 4 presents information related to the decomposition of the correlation coefficients, representing the relations between yield and morphological traits, into direct and indirect in the complex stable line L 6.3.3. It was found that the length of the twelfth leaf (29.73%) had the strongest overall positive effect on the yield, followed by the width of the twelfth leaf

(23.81%), the length of the sixth leaf (21.71%). The high correlation coefficient between the length of the twelfth leaf and the yield is largely due to the strong indirect influence between them. The negative indirect effect of the length of the sixth leaf is weak and it is largely compensated by the direct effect of this feature.

The presence of statistically proven correlation coefficients is a prerequisite for the application of multiple regression analysis in order to model the relationships between yield and some morphological features. The obtained statistically reliable model is:

$$y = -170.631+4.733x_3-2.350x_4+7.275x_5-3.727x_6.$$

Table 4. Direct and indirect influences of the studied traits on the yield from line L 6.3.3

Trait	Direct coefficient (Beta)	Indirect(Path) coefficient	Correlation coefficient (R)
Height ( $x_1$ )	-0.574	1.087	0.513
Number of leaves ( $x_2$ )	1.248	-0.960	0.288
Length of the 6th leaf ( $x_3$ )	0.916	-0.135	0.781
Width of the 6th leaf ( $x_4$ )	-0.635	1.226	0.591
Length of the 12th leaf ( $x_5$ )	0.306	0.698	0.914
Width of the 12th leaf ( $x_6$ )	0.484	0.334	0.818

## CONCLUSIONS

The study of the traits influencing the productive potential of complex sustainable to both viral diseases tobacco lines is essential for the further correct and effective planning of the experimental work and the selection programs. As a result of the applied correlation analysis at line L 5, a significant positive relation between the yield and the height of the plant is established, as well as a very strong, positive correlation with the number of leaves, the sizes of the sixth and twelfth leaves. For line L 6.3.3, the yield is in a significant positive relation with the length of the sixth leaf, moderate relation with the width of the sixth leaf and in a strong, positive relation with the length and width of the twelfth leaf. In L 6.3.3, the number of leaves and the length of the sixth leaf have the highest direct effect on yield.

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## MODELS OF QUANTITATIVE ESTIMATION OF SOWING DENSITY EFFECT ON MAIZE YIELD AND ITS DEPENDENCE ON WEATHER CONDITIONS

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### Abstract

*This paper is focused on dependence of corn yield on plant density at harvest time and the influence of weather conditions during crop growth period on the parameters of yield-factor response models. It is established that the main parameters of the models are significantly influenced by both moisture supply (precipitation and water productivity in the soil) and hydrothermal conditions (hydrothermal coefficient and moisture coefficient). The 2016-2018 years are characterized by quite close mathematical relationship ( $R^2=0.9672-0.99940$ ) between the corn grain yield and sowing density. It was found the existing dependence of the model parameters on weather conditions and sufficiently high resistance of hybrid DS0493B to external conditions, since the deviation of the values of these conditions has turned out to be much greater than the deviation of the model parameters.*

**Key words:** maize yield, sowing density, crop modeling, quantitative dependence, water consumption.

### INTRODUCTION

In recent decades, the yield of corn hybrids has increased significantly due to the use of new genotypes and intensification of plant growing technologies (Assefa et al., 2018; Kaminskyi & Asahishvili, 2020). There is no doubt that the density of plants is the main technological factor in the formation of yields, and the optimization of growing conditions of one cultivar or another makes it possible to increase the productivity of both one plant and sowing as a whole (Sarlangue et al., 2007; Liu Y. et al., 2021). This is due to the fact that since the formed yield is a product of the plant photosynthetic activity, it is conceivable that the corresponding area of the leaf surface is formed with each sowing density that is the basis of the process of photosynthesis. It is understood that its intensity will be determined by the above optimization measures. Thus, it can be argued that the density of plants in the field is the basic condition that determines the leaf area duration of the crop, according to the existing conditions of cultivation, the intensity of photosynthesis, and hence the final yield of

one plant and sowing (Shapiro & Wortmann, 2006). On the other hand, it is generally known that in sowing there is the competition among plants for space, light, water, mineral nutrients and carbon dioxide (Attia et al., 2021).

There is no doubt that in this case we are talking about clean crops field without weeds, as in other cases there is the competition among crop and weeds (Baer et al., 1984; Mischenko et al., 2019), which are not the subject of study in this work. Having regard to the above, we may talk of the optimal value of plant density. At the same time, according to the rules of agriculture this value is not constant, but depends on the specified growing conditions (moisture, heat, mineral elements, etc.) and the characteristics of the variety or hybrid of this crop (Kharchenko et al, 2019). All the above mentioned reasonably confirm the continued expedience of studying this issue in different soil and climatic zones under different conditions of moisture and fertilizers, new varieties or hybrids, which in general is the basis of varietal farming (Ren et al., 2021; Sher et al., 2017; Yan et al, 2021).

## MATERIALS AND METHODS

When examining the above problem in reasonable detail, we should consider the dependence of the yield of one plant ( $Y_p$ , g) and the entire sowing ( $Y_s$ , t/ha) depending on its density ( $X$ , thousand pcs/ha), which is shown in Figure 1 (Kharchenko, 2003).

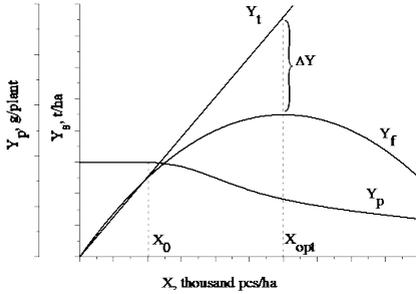


Figure 1. General scheme of dependence of the main crop yields on sowing density

Thus, with certain basic density ( $X_0$ ), there is no competition in sowing, the yield of one plant has a maximum value, and the yield of the entire sowing may be described by the straight-line dependence of the following type:

$$Y_s = Y_p \cdot X_0 \cdot 10^{-2}, t/ha \quad (1)$$

Further growth in density results in competition, and the closer the sowing is, the more intensive the competition is. The yield of one plant decreases sharply, and the yield of the entire sowing begins to be described by curvilinear dependence. At the same time, the higher the density is, the greater the effect of competition is, and therefore, the greater the deviation of the actual yield from direct dependence is (Fig. 1). It is known that when assessing the yield of the main products (grain, roots), the dependence of yield on density is characterized by a single-humped dome-shaped curve with a salient optimal value of this density. This is due to the fact that if density is more than optimal, the yield of one plant decreases more than the density increases. With some approximation, this dependence may be described by a quadratic parabola without a free term:

$$Y = aX^2 + bX, t/ha \quad (2)$$

where:  $a$  and  $b$  are empirical coefficients.

Thus, it can be argued that maximum productivity is not formed at the highest value of yield of one plant, but in the conditions when the product of the mass of one plant and density becomes the maximum value. It is clear that this is determined by the nature and intensity of reduction in the mass of 1 plant with increasing density that in turn depends on the competitive ability of sowing itself.

Therefore, in the case of competition in sowing, the actual crop yield is described by curvilinear dependence, and one of the key characteristics of this model is the optimal density value, that is, the density, at which the maximum yield in this series is formed (Fig. 1):

$$X_{opt} = -\frac{b}{2a}, \text{ thousand spcs./ha} \quad (3)$$

Another important parameter of this model is the value of maximum yield:

$$Y_{max} = aX_{opt}^2 + bX_{opt}, t/ha \quad (4)$$

Another characteristic indicator of this model, as mentioned above, is the highest density, at which competition in sowing is not observed ( $X_0$ ) yet and which in general may be the subject of study. At the same time, hypothetically, without competition in sowing, with the known or accepted value of  $X_0$ , the theoretical yield ( $Y_t$ ) may be determined by the straight-line dependence (Fig. 1):

$$Y_t = (aX_0 + \epsilon), \text{ } \bar{\tau} t/ha \quad (5)$$

The difference ( $\Delta Y$ ) between the maximum yield ( $Y_{max}$ ) and the theoretical yield ( $Y_t$ ) with the optimal density ( $X_{opt}$ ) enables to determine the indicator of the effect of competition in sowing on the formation of yield ( $S$ , %) with respect to the theoretical value:

$$S = \frac{100a(X_0 - X_{opt})}{(aX_0 + \epsilon)}, \% \quad (6)$$

It is clear that the value  $(100 - S)$  is the competitive ability of sowing of this variety in specific weather and technological conditions.

The field experiments on studying the effect of the sowing density on the crop yield were conducted on the experimental fields of the Institute of Agriculture of the North-East of NAAS during 2016 – 2018. The soils were chernozem leached middle loamy on loess with the following basic characteristics: humus content was 4.1–4.7 %,  $pH_{KCl} = 5.0$ , easily

hydrolysable nitrogen content (by Kornfield) was 112.0, and movable compounds of  $P_2O_5$  and  $K_2O$  (by Chirikov) were 118.0 and 100.0 mg/kg, respectively.

The replication of experience was triple. The area of the experimental plot was 28 m<sup>2</sup>. The yield was taken into account at humidity of 14%.

The study was conducted with the hybrid DS0493B, which is a product of Dow Seeds Company. The recommended harvesting density is 50-65 thousand plants/hectare (unfavorable conditions); 75-90 thousand plants/hectare (favorable conditions). Yield potential: 15 t/ha.

## RESULTS AND DISCUSSIONS

Nowadays, there are many models for predicting the yield of agricultural crops, in particular, corn (Kharchenko et al., 2019). Using linear regression to determine the response to changes in planting density in maize, Assefa et al. (2018) reported that the contribution of plant density to yield increases ranges from 8.5 to 17 %. Using the Bayesian computational methods, Lacasa et al. (2021) have established a significant relationship between crop yield and geographic location, while the planting density and the value of the economic efficiency of growing corn have changed.

Newer corn hybrids have a significant response to nitrogen fertilizers (Sher et al., 2017; Asanishvili et al., 2020; Ciampitti et al., 2021). contents that hybrids with low FAO and lower plant height tolerate high plant densities, increasing the number of grains per area. Taller plants can lodge in dense plantings, and with lower densities, at the same time the diameter of the stem and shoot dry weights increase. The response of hybrids to environmental factors can also be predicted using cluster analysis and cluster diagrams (Palamarchuk et al., 2021).

According to Li et al. (2015), growing corn with drip irrigation and plastic mulching, the yield index, dry matter strongly depended on the plant density  $\leq 4.7$  plants per m<sup>2</sup>. With an increase in the density of 8.3 plants per m<sup>2</sup>, a relationship with the yield index has not been established, but there is an effect on grain yield and dry matter. With an increase in the density

of 10.7 or more per m<sup>2</sup>, no effect on dry matter has been established, but there was a relationship with a yield index.

Sun et al. (2016), using the APSIM model, which takes into account the variability of weather factors, obtained good predictability of grain yield and corn biomass. According to the data of 11 years of field experience, it has been confirmed that plant density is one of the most important factors that affect corn yield, while plant density correlates with the optimal sowing time.

Shahhosseini et al. (2021) used different calculation models (linear regression, LASSO, LightGBM, random forest, XGBoost) for predicting yields in the corn belt USA. They found that soil moisture is the most influential factor that affects plant phenology. It was proposed to use in modeling such factors as soil water during the growing season and average water table.

Attia et al. (2021) used multivariate analysis, variance decomposition method, Sobol method and proposed their own model for predicting yield. They found that plant biometrics and corn yield were significantly correlated with soil hydrological characteristics, soil fertility, organic carbon content and the phenology of corn depended on the genotype.

Jiang et al. (2021) proposed a new model with spatiotemporally varying coefficient (STVC) that takes into account the spatio-temporal non-stationarity and improves the explanation of the influence of meteorological data on the yield of corn. Khanal et al. (2021), using high-resolution maps with remote sensing technology and modern forecasting equations, has also developed a model that could be applied to access the effect tillage, fertilization and yield with high accuracy.

Pepeliaev et al. (2020) propose a quantile regression method for yield modeling depending on climatic parameters for the central region of Ukraine. NDVI with polynomial regression analysis, Slant Range, Mathcad, stress index is also used for accurately crop yield predicting (Pasicznyk et al., 2019; Pasicznyk, 2020; Serdiuchenko et al., 2019). NDVI recommended to be determinate during silking (VI) and flowering (R1) (Vozhehova et al., 2020).

The use of different models for predicting corn yields depending on weather conditions shows large discrepancies that need to be processed by a larger database (Yin & Leng, 2021; Drobotko et al., 2020).

The regression and correlation analysis in our research has made it possible to form mathematical models of yield dependence on sowing density and to determine their reliability (Table 1).

Table 1. Effect of sowing density on the grain yield of DS0493B maize hybrid

Year	Density, thousand pcs/ha		Actual yield (average), t/ha	Yield-density model	Determination coefficient, R <sup>2</sup>
	after germination	during harvesting			
2016	60	57.1	11.8	Y = -0.00202X <sup>2</sup> + 0.30978X	0.96720
	70	61.4	10.7		
	80	71.4	11.7		
	90	81.4	12.0		
2017	60	56.1	10.5	Y = -0.00233X <sup>2</sup> + 0.32008X	0.99940
	70	64.6	11.1		
	80	78.4	10.8		
	90	82.2	10.5		
2018	60	56.5	9.2	Y = -0.00209X <sup>2</sup> + 0.27220X	0.98575
	70	62.6	8.4		
	80	77.7	8.2		
	90	85.1	8.3		

A graphic illustration of the obtained models is shown in Figure 2.

These data indicate a significant difference between the above parameters of the generated models. Thus, if in the conditions of 2016 the optimal density ( $X_{opt}$ ) and the maximum yield ( $Y_{max}$ ) had the largest values, in 2018 – the lowest (respectively 76.7 and 65.1 thousand pcs/ha and 11.88 and 8.86 t/ha) with an intermediate value in 2017 (Table 2).

On average, over the three years the optimum plant density amounted to 70.2 thousand pcs/ha and the maximum yield was equal to 10.58 t/ha.

Usanova et al. (2019) reports the optimal data and the highest net income was obtained at a plant density of 100 thousand plants per hectare. Different hybrids respond differently to changes in environmental and soil conditions (Usanova & Migulev, 2019). Semina et al. (2018) results show that the greater the plant density, the lower the amount of grain in the cob (by 8.1-21.1% - on leached heavy loamy chernozems).

The problem of uncertainty of the value of the basic density ( $X_0$ ) rises from the estimation of the indicator of competition in sowing (S, %),

or competitive ability of sowing (100-S). Since this value has not been studied experimentally, this paper contains the proposal that its analytical determination should be made provided that at optimal density the competition indicator is about 45% (Kharchenko, 2003).

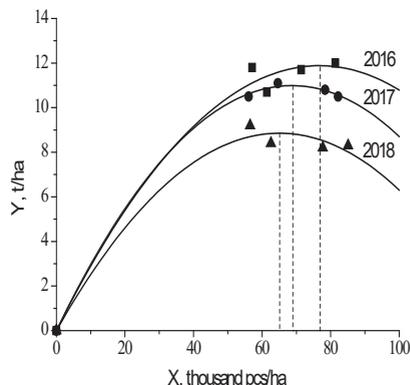


Figure 2. Models of response of yield of hybrid maize DS0493B on plant density by the study years

$$\begin{aligned}
 2016: & \quad Y = -0.00202 X^2 + 0.30978X & \quad R^2 = 0.98729 \\
 2017: & \quad Y = -0.00233 X^2 + 0.32008X & \quad R^2 = 0.99940 \\
 2018: & \quad Y = -0.00209 X^2 + 0.27220X & \quad R^2 = 0.98575
 \end{aligned}$$

Table 2. Parameters of Yield – Density Models of DS0493B maize hybrid (according to the data obtained during 2016-2018)

Indicators	Years			Average
	2016	2017	2018	
Optimal sowing density ( $X_{opt}$ ), thousand pcs/ha	76.7	68.7	65.1	70.2
Maximum yield ( $Y_{max}$ ), t/ha	11.88	11.00	8.86	10.58
Basic sowing density, thousand pcs/ha (at S = 45%)	13.95	12.53	11.84	12.77

Thus, the dependence 6 results in the following:

$$X_0 = \frac{100a \cdot X_{opt} + P \cdot B}{(100 - P)a}, \text{ thousand } \varrightarrow \text{ pcs/ha} \quad (7)$$

The calculations have shown that the density of sowing, at which competition begins, ranges from 13.95 thousand pcs/ha (2016) to 11.84 thousand pcs/ha (2018) with the average value of 12.77.

All of the above suggest significantly different conditions for the formation of yield in those years. At the same time, there is no doubt that since the main parameters of the models had the best values in the conditions of 2016, these

conditions may be considered to be the most favorable.

It is well known that one of the main factors of crop growth and development and, therefore, the formation of yield, or weather conditions, is the moisture resource. At the same time, the estimation of availability of this resource may be carried out both by the growing season of the crop and its individual parts (Polevoy, 2007). It is the estimation of the conditions by the provision of moisture resources that may be made both in terms of precipitation amount and the full resource of this factor that is the amount of precipitation for a specific period (A, mm) and moisture reserves (productive) in the established or accepted soil layer at the beginning of this period (MP, mm).

It has been established that the conditions of natural moisture of the vegetation periods of maize during the study years are significantly different. Thus, according to the amount of precipitation at a rate of 254 mm (Logvinova et al., 1976), the actual values ranged from 403.7 mm in 2016 to 118.7 mm in 2018 with a deviation from the norm of +148.3 to -135.3 mm. The moisture reserves in the meter layer of soil during germination were 175.0-36.6 mm with an average value of 154.0 mm (Logvinova et al., 1976). In general, the moisture provided to the crop (initial moisture reserves together with the actual precipitation) for the years ranged from 578.7 to 271.1 mm, that is, the deviation from the long-term average annual (408.0) ranged from +170.7 to -137.9 mm.

There is no doubt that the estimation of the conditions for the formation of the maize yield cannot be complete without taking into account the resource of such factor as heat, which is determined by the sum of active air temperatures. In addition, it is notorious that a significant indicator of the characteristics of the growing season is hydrothermal conditions, which includes various combinations of moisture and heat resources. The most common indicators of hydrothermal conditions are the hydrothermal coefficient of Selianinov H. T. (HTC) (Logvinova et al., 1976) and the coefficient of moisture of Bova N. V. (Chirikov, 1986). Thus, it is established that with average long-term value of HTC equal to 1.13 for the years of research it was 0.91 with fluctuations during the years from 1.62 to 0.44.

Therefore, the above makes it possible to assert that the weather conditions of 2016-2018 were somewhat drier than normal, and the actual conditions ranged from wet (2016) to very dry (2017 and 2018). Similarly, the conditions are characterized by the coefficient of moisture. The results of comparison of the key characteristics of the proposed models and various indicators of the characteristics of the conditions are given in the Table 3.

Table 3. Comparative characteristics of the basic parameters of the Yield – Density Model and indicators of the conditions of the maize growing season

Indicators	Years			Average value for three year	Long-term average annual
	2016	2017	2018		
Optimal sowing density ( $X_{opt}$ ), % in relation to 2016	100	89.6	84.9	–	–
Maximum yield ( $Y_{max}$ ), % in relation to 2016	100.0	92.6	74.6	–	–
Basic sowing density, % in relation to 2016	100	89.8	84.9	–	–
Precipitation for May - August ( $\Sigma A$ ), mm	403.7	157.2	118.7	226.5	254.0
In relation to 2016, %	100.0	38.9	29.4	–	–
Proposed moisture resource ( $\Sigma A + MP$ ), mm	578.7	293.8	271.1	381.2	408.0
In relation to 2016, %	100.0	50.7	46.8	–	–
Sum of active temperatures ( $\Sigma t > 0C$ )	2487	2332	2680	2500	2241
In relation to 2016, %	100.0	93.8	107.8	–	–
Hydrothermal coefficient ( $HTC = \frac{\Sigma A}{0.1 \Sigma t}$ )	1.62	0.67	0.44	0.91	1.13
In relation to 2016, %	100.0	41.3	27.2	–	–
Coefficient of moisture ( $Cm = \frac{\Sigma A + MP}{0.1 \Sigma t}$ )	2.32	1.26	1.01	1.52	1.82
In relation to 2016, %	100.0	54.3	43.5	–	–

Comparing the fluctuations of the basic parameters of the Yield – Density Model by years and the actual conditions of the growing period of these years, it can be argued that there is a fairly close relationship between them. However, it should be noted that this hybrid maize is relatively resistant to changes in weather conditions. Thus, if the deviation of the values of the established parameters of the conditions by years from the conditions of 2016 range from 51.2% (A+MP) to 72.8 (HTC), the fluctuations of the model parameters do not exceed 25.4 ( $Y_{max}$ ).

So, the climate is changing and the analysis of parameters of soil temperature, humidity, precipitation should be taken into account in planning crop yields (Maltais-Landry & Lobell,

2012; Rusu et al., 2013; Iizumi, 2017; Maxim et al., 2018; Pasca & Rusu, 2018).

## CONCLUSIONS

Regression and correlation analysis allowed to make mathematical models of yield dependence on sowing density and to determine their reliability. A close mathematical relationship of reaction of maize grain yield to plant density ( $R^2 = 0.9672 - 0.99940$ ) was observed during all the years of research.

The sowing density at which competition begins ranged from 11.84 to 13.95 thousand units/ha in 2016-2018 (in average 12.77). Rather close dependence of parameters of the specified models on weather conditions of the crop growing period has been determined.

It is proved that under the conditions of 2016-2018, the hybrid maize DS0493B turned out to be quite resistant to external conditions, since the deviation of values of these conditions was much greater than the deviation of parameters of the models.

The proposed models could help in determination of the impact of competition between plants on the yields, calculate the optimal value of the density at which the maximum yield is formed.

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## COMPARATIVE BIOCHEMICAL AND TECHNOLOGICAL STUDIES OF BULGARIAN DURUM WHEAT GRAIN

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### Abstract

*During the period 2015-2018 in the Educational Experimental and Implementation Base of the Department of Plant Breeding at the Agricultural University - Plovdiv a comparative study of the biochemical and technological properties of the grain of the Bulgarian varieties of durum wheat Progress (standard), Beloslava, Vazhod and Yavor was conducted. The field experiment is based on the rapeseed predecessor by the block method in four repetitions with the size of the harvest plot - 15m<sup>2</sup>. As a result of the experiment, the following was found: Durum wheat variety Yavor is characterized by the highest content of crude protein (14.8%) in the grain, as well as common (13.5%) and essential (3.61%) amino acids in % to absolute dry matter compared to the standard. The highest yield of wet gluten was reported in the varieties Beloslava (42.9%) and Vazhod (40.3%), as well as the highest number of chebobacterial strength and sedimentation number compared to the variety Progress.*

**Key words:** durum wheat, varieties, biochemical and technological properties.

### INTRODUCTION

Durum wheat is the main raw material for the production of pasta, due to its valuable biochemical and technological properties of grain, which allow to obtain high quality products. The main quality indicators of durum wheat varieties - protein content and gluten strength are factors for the quality of prepared pasta. Pasta with a bright yellow colour and good cooking quality (Martí et al., 2016; Sayaslan et al., 2012; Kolev et al., 2008) is obtained from varieties that combine high yellow pigments (over 7 ppm), protein concentration (over 15-16% dm), and strong gluten.

The interaction of a complex of factors, including variety, ecological conditions, and cultivation technology, is crucial for improving the quality of durum wheat grain (Haile et al., 2018; Delibaltova & Kirchev, 2010; Kirchev, 2011; Kolev et al., 2008). Optimizing these factors leads to high results in durum wheat production (Yanev & Kolev, 2008). Each variety with its genetic capacity is an effective means of increasing grain yield and quality (Kolev et al., 2004; Yanev, 2006; Yanev et al., 2008; Kolev et al., 2011). By growing varieties suitable for a particular region, we can reduce

the negative impact of environmental conditions (Clarke, 2000; Mangova and Petrova, 2007; Kolev et al., 2012).

The aim of the present study is to establish the biochemical and technological properties of grain of Bulgarian durum wheat varieties.

### MATERIALS AND METHODS

During the period 2015-2018 we conducted a field experiment in the Educational, Experimental and Implementation Base of the Agricultural University Plovdiv, using the block method, in four repetitions with the size of the harvest plot of 15 m<sup>2</sup>.

The varieties Progress (standard), Beloslava, Vazhod, and Yavor were tested.

The durum wheat varieties were grown in a crop rotation with rapeseed. Depending on the climatic conditions, immediately after harvesting the predecessor, the plant remains were removed, and the soil was disc-processed two or three times with disc harrows at a depth of 8-12 cm. The sowing of the studied varieties of durum wheat was carried out in the optimal period for Southern Bulgaria from 20<sup>th</sup> October to 5<sup>th</sup> November with a sowing rate of 500 germinating seeds of m<sup>2</sup>.

The total amount of phosphorus P<sub>8</sub> kg/da is applied with the main tillage, and nitrogen fertilizer (ammonium nitrate) N<sub>12</sub> - 1/3 before sowing, and the remaining amount 2/3 in early spring as fertilizer.

Weed, disease and pest control is carried out according to the established technology of growing durum wheat (Yanev et al., 2008). Harvesting is done in full maturity, by direct harvesting with a small experimental Wintersteiger seedmaster universal combine harvester.

The chemical and technological analyses were performed at the Laboratory Testing Complex at Agricultural University Plovdiv.

The grain samples from the tested varieties of durum wheat are qualified according to the indicators: mass per 1000 grains under BDS ISO 520: 2003; hectolitre mass under BDS ISO 7971-2: 2000; grain vitreousness under BDS EN 15585: 2008; sedimentation number of the flour by the method of Pumpyansky; extraction of wet and dry gluten; gluten loosening; baking value (BV) according to BDS EN ISO 21415-2: 2008, and fermentation index according to the Pelshenke method.

The determination of the total nitrogen in the grain was performed in ground samples of wheat grain after wet burning (Gorbanov, 1990) with concentrated sulfuric acid and hydrogen peroxide. We calculated the crude protein based on the fact that the nitrogen content in the wheat grain protein is 17.5%. The percentage of nitrogen determined by the described method was multiplied by a factor of 5.7.

We carried out the quality and quantity determination of the total amino acids in the grain after acid hydrolysis with 6 and HCL for 24 hours at 105°C in a thermostat. The reading was done on an automatic amino analyser, manufactured in the Czech Republic.

The statistical processing of the data obtained on the studied indicators was performed with the software BIostat (Penchev, 1998).

## RESULTS AND DISCUSSIONS

The amount of precipitation during the vegetation of durum wheat during the years of the experiment is as follows: 2015/2016 - 396.5 mm/m<sup>2</sup>, 2016/2017 - 278.3 mm/m<sup>2</sup>, 2017/2018

- 457.2 mm/m<sup>2</sup>, with a climatic norm of 419.6 mm/m<sup>2</sup>. The amount of precipitation in the second experimental year is less than the climatic norm, but due to their better distribution during the critical stages of plant development, the harvest year of 2016/2017 is more favourable for the growth and development of durum wheat.

Unfavourable for the development of plants is the harvest year 2015-2016 due to the lower rainfall in the period from February to June compared to the climatic norm, which had a negative impact on the productivity of durum wheat (Figures 1 and 2).

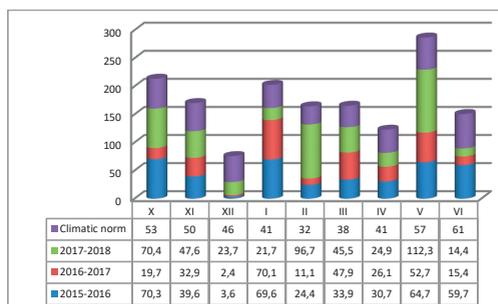


Figure 1. Precipitation by months, sum mm/m<sup>2</sup>

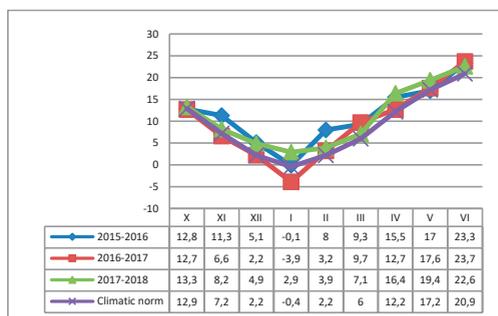


Figure 2. Monthly temperatures (average)

### *Influence of the variety on some physical indicators and wheat grain yield*

The physical properties of durum wheat grain are determined by the variety and the growing conditions. These properties are significantly influenced by meteorological and soil conditions. Agrotechnical factors do not affect equally. Stronger changes are caused by nitrogen fertilization and irrigation, which improve some and worsen other physical properties.

The mass of 1000 grains characterizes the well-nutrition and grain size, and is an indirect milling indicator of the output of the obtained flour when milling the grain.

From the studied varieties of durum wheat the standard Progress variety has the highest mass per 1000 grains (Table 1) with an absolute weight of 59.6 g. The other varieties have lower values of mass per 1000 grains compared to the standard.

Another indirect indicator of the milling properties is grain hectolitre mass. When buying wheat, the milling properties are evaluated per hectolitre mass. It is determined by the quality and condition of the grain - moisture, culture and inert impurities, size and shape. It is positively influenced by agro technical factors such as fertilization and predecessor.

Table 1. The influence of variety on the physical properties and grain yield of durum wheat

Varieties	The physical properties						Grain yield	
	Mass per 1000 grains		Hectolitre mass		Vitreousness		kg/da	%
	g	%	kg	%	%	% to st.		
1. Progress	59.6	100.0	81.6	100.0	92.1	100.0	383.5	100.0
2. Beloslava	55.4	93.0	82.7	101.3	98.6	107.1	423.7	110.5
3. Vuzhod	58.1	97.5	82.9	101.6	97.9	106.3	458.0	119.4
4. Yavor	57.9	97.1	82.0	100.5	94.1	102.2	395.8	103.2
GD 5%	4.12	6.9	4.12	5.1	5.7	6.1	35.1	9.1

All tested varieties have a mathematically unproven higher grain hectolitre mass compared to the Progress variety.

Vitreousness is also an important milling indicator and its high values determine the production of white flours with high yield. This indicator strongly correlates with the content of protein and starch. It is influenced by many factors, but the varietal characteristics, fertilization and meteorological conditions during the formation and ripening of the grain are decisive. For the studied varieties vitreousness varies from 92.1% for Progress variety to 98.6% for Beloslava variety and the value of this indicator is by 7.1% higher than the standard.

According to Filipov (Filipov & Mangova, 1992), in dry years vitreousness of wheat grain is most affected by nitrogen fertilization, followed by the varietal characteristics.

The yields of durum wheat grain depend on many factors, but the most important of them are the position of the crop in the crop rotation, the stocks of the available to the plants forms of nitrogen, phosphorus and potassium, the fertilization rates, the times of fertilizer

application, the climatic conditions during vegetation, etc.

The highest grain yield during the study period was obtained from the Vazhod variety. The grain obtained from this variety is by 74.5 kg/da (19.4%) more than the standard Progress variety. Beloslava variety follows by 40.2 kg/da (10.5%) more than the standard. Third, in terms of yield is Yavor variety with grain addition of 12.3 kg/da (3.2%).

The increase in yield is mathematically proven for varieties Vazhod and Beloslava, while in Yavor variety higher productivity than the standard is mathematically unproven.

### *Influence of the variety on some chemical parameters of wheat grain*

Nitrogen and variety are among the main factors that, in addition to the yield, have a strong influence on the quality of wheat.

Protein content is one of the most important indicators characterizing the quality of wheat grain. According to a number of authors, grain yield and protein concentration cannot be improved simultaneously. Achieving a high protein content in the grain can indirectly affect the yield, i.e. there is an inverse relationship between grain yield and protein concentration. The importance of protein content for the technological qualities of wheat is direct and indirect. Gluten production is closely correlated with it.

The analysis of the obtained results for the content of crude protein in the grain shows that Yavor variety is characterized with the highest protein content, and this increase is by 3.5% for Yavor compared to Progress variety standard. In varieties Beloslava and Vazhod it is by 15.4% to 19% lower (Table 2).

The results obtained confirm the established inverse relationship between grain yield and protein concentration in it.

Table 2. Content of total nitrogen and crude protein in durum wheat grain

Varieties	Absolute dry matter %	Content of total nitrogen %	Crude protein	
			N,5,7	%
1. Progress	90.05	2.51	14.3	100.0
2. Beloslava	88.2	2.14	12.1	84.6
3. Vuzhod	88.1	2.03	11.6	81.1
4. Yavor	87.9	2.60	14.8	103.5

A similar pattern is observed in the analysis of data on the amount of total amino acids in the grain of the studied varieties (Table 3).

Table 3. Content of total amino acids in durum wheat grain

Varieties	In % to absolute dry matter			
	Progress	Beloslava	Vuzhod	Yavor
<b>Amino acids</b>				
<b>Monoaminoacarbonates</b>				
Glycine	0.54	0.41	0.43	0.50
Alanine	0.55	0.44	0.43	0.52
Valin	0.50	0.44	0.45	0.53
<b>Dicarbonates</b>				
Aspartic acid	0.78	0.58	0.72	0.90
Glutamic acid	4.22	4.21	3.82	4.54
<b>Oxyaminocarbons</b>				
Serine	0.63	0.58	0.64	0.61
Threonine	0.39	0.35	0.32	0.39
<b>Basic</b>				
Lysine	0.51	0.36	0.43	0.47
Histidine	0.35	0.26	0.32	0.34
Arginine	0.75	0.63	0.65	0.71
<b>Sulfur-containing</b>				
Methionine	0.13	0.14	0.09	0.14
Cystine	0.12	0.11	0.14	0.14
<b>Aromatic</b>				
Phenylalanine	0.65	0.62	0.58	0.73
Tyrosine	0.28	0.27	0.25	0.31
Proline	1.41	1.45	1.32	1.51
<b>Leucine</b>				
Leucine	0.84	0.80	0.80	0.92
Isoleucine	0.41	0.37	0.37	0.43
<b>Total:</b>	<b>13.06</b>	<b>12.12</b>	<b>11.62</b>	<b>13.50</b>
<b>%</b>	<b>100.0</b>	<b>92.8</b>	<b>89.0</b>	<b>103.3</b>

In the case of Yavor variety the concentration of total amino acids in the grain is 3.3% higher than in the standard, while in the case of Beloslava and Vuzhod varieties the decrease of this indicator is 7.2% for Beloslava and 11.0% for Vuzhod, where this decrease is in all groups of amino acids. A similar trend is observed in the amount of essential amino acids in the grain of the studied varieties (Table 4).

Table 4. Content of essential amino acids in durum wheat grain

Varieties	In % to absolute dry matter			
	Progress	Beloslava	Vuzhod	Yavor
<b>Amino acids</b>				
Lysine	0.51	0.36	0.43	0.47
Threonine	0.39	0.35	0.32	0.39
Valine	0.50	0.44	0.45	0.53
Methionine	0.13	0.14	0.09	0.14
Leucine	0.84	0.80	0.80	0.92
Isoleucine	0.41	0.37	0.37	0.43
Phenylalanine	0.65	0.62	0.58	0.73
<b>Общо:</b>	<b>3.43</b>	<b>3.08</b>	<b>3.04</b>	<b>3.61</b>
<b>%</b>	<b>100.0</b>	<b>89.8</b>	<b>88.6</b>	<b>105.2</b>

### *Influence of the variety on the technological properties of wheat grain*

One of the most important indicators of wheat quality is the quantity and quality of gluten. According to a number of authors, the quality of wheat depends not so much on the amount of crude protein, but above all on the quality of gluten, which is closely related to the type, variety, etc.

Wet gluten is an extremely important indicator for both wheat grain and its products - flour, dough, bread, etc. It is a known fact from literature resources that it is positively correlated with crude protein.

Gluten is a mixture of proteins. It is an elastic, tough and water-insoluble substance. It contains gliadin (44%), glutenins (41%), and other protein substances (15%). On its physical properties - elasticity and extensibility, depend the elasticity, porosity, gas retention when baking bread, the volume and duration of its storage.

Wet gluten is a complex colloidal system of two main protein substances: gliadins (soluble in alcohol) and glutenins (insoluble). It is obtained after prolonged washing of the dough with water or saline solution. It is done with the help of a gluten washer until a yielding-elastic substance is obtained, which is no longer washed away. The main indicators of the physical condition of wet gluten are its toughness, extensibility, and cohesion.

It is found that the amount of protein and gluten depends on 70% of the ecological conditions and the cultivation technology and 30% on the variety.

One of the important indicators for the quality of wheat is the quantity and quality of gluten - wet and dry (%). The baking and pasta qualities of wheat largely depend on the physico-chemical properties of gluten, its quality - on the variety, ecological conditions and technology of wheat cultivation, and especially on fertilization, and late leaf nutrition after ear formation of the crop, the timing and method of harvesting, drying, preparation and storage and is of particular importance for the production of bread, pasta and confectionery.

According to BDS, the content of wet gluten in the grain of strong and durum wheat must be over 28%. Its quantity depends more on the consistency than on the grain size. Only with the same consistency does the larger grain contain more gluten. To a large extent the quality of gluten depends on the temperature and humidity of the air in the filling and ripening phase of the grain. At lower temperatures and higher humidity, grain with a lower content and quality of protein and gluten is obtained.

The content of wet gluten is higher in the varieties Beloslava by 15.3%, and Vuzhod by 8.3% compared to Progress variety, but gluten in these varieties is of poorer quality, for which we judge by the loosening of gluten (Table 5).

Table 5. Influence of the variety on some technological properties of grain

Varieties	Wet gluten		Dry gluten		Sedimentation number (sm <sup>3</sup> )	Gluten release (mm)	Number of baking power usl. ed
	%	% кбМ st	%	% to st,			
1.Progress	37.2	100.0	13.8	100.0	51	2.1	66
2.Beloslava	42.9	115.3	14.9	108.0	56	2.9	78
3.Vuzhod	40.3	108.3	14.4	104.3	55	2.6	75
4.Yavor	36.9	99.2	14.0	101.4	49	2.3	64
GD 5%	2.94	7.9	0.98	7.1			

Progress and Yavor varieties have a relatively lower gluten content, but their gluten is firm, inelastic, with minimal loosening of the gluten ball (2.1 and 2.3 mm), which is an indication of strong gluten. The best combination between quantity and quality of gluten is observed in Beloslava and Vazhod varieties. The high baking value (78 and 75) conditional units is an indication of good gluten quality.

The baking value is a set of indicators for the quantity and quality of gluten. All studied varieties are characterized by high baking values. A similar trend is observed with regard to the amount of dry gluten in the grain of the tested varieties of durum wheat. Beloslava variety is characterized by the highest content of dry gluten - 14.9% or by 8.0% higher than Progress variety. In other varieties, an increase in dry gluten is also observed, but it is mathematically unproven.

Sedimentation is a method for determining the puffiness of flour. The sedimentation number of common wheat has a positive correlation with its physical, chemical-technological, and baking properties. It gives an indirect assessment of the baking value. The sedimentation number is positively related to crude protein and wet gluten and is a swelling of gluten proteins in a dilute acid solution. In our studies Beloslava and Vazhod varieties are distinguished by a high sedimentation number.

## CONCLUSIONS

For the first time comparative studies have been made on the influence of varietal characteristics on the chemical composition and

technological properties of the grain of four varieties of durum wheat grown in Southern Bulgaria.

Of the studied varieties of durum wheat Progress variety has the highest mass per 1000 grains, while the other varieties have lower values of this indicator. All tested varieties have a mathematically unproven higher hectolitre grain mass compared to the Progress variety standard.

Under the ecological conditions of Plovdiv region, the highest grain yield is obtained from Vazhod variety durum wheat. The grain obtained from this variety is by 74.5 kg/da (19.4%) more than the standard Progress variety.

The grains of Yavor variety are characterized by the highest protein content, as this increase is 3.5% more than the standard, while the amount of protein in the other two varieties is lower.

The highest concentration of total and essential amino acids is in the grain of Yavor variety, while in the other two varieties it is significantly lower than the standard.

The highest yield of gluten was reported in the varieties Beloslava and Vazhod, as well as the highest baking value and sedimentation number compared to Progress variety.

The tested varieties of durum wheat have good physicochemical indicators and productive possibilities and can be successfully grown under the ecological conditions of Southern Bulgaria.

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## SCIENTIFIC RESULTS OF ETCHED GRAIN SEEDS MACHINE

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### Abstract

*The article poses the main problem of the loss of seed germination during its storage in granaries. During storage, microorganisms multiply in grain, which not only worsen the grain yield, but also reduce the quality of the product. The most harmful and resistant species are found among the pests of grain stocks. The chemical method is one of the effective measures for pest control - which is the disinfection of seed material from external and internal infection, as well as pathogens of various bacterial and fungal diseases, spreading through seeds, soil and planting material. Such treatment protects crops from damage, contributing to the preservation of up to 30-50% of the crop. The equipment used in organizations for seed treatment does not always meet modern agrotechnical requirements; therefore, the authors have developed a technological scheme and design of an installation for disinfecting grain in a stream when storing it. The test results of the proposed technical device allow us to conclude about the appropriateness of its use.*

**Key words:** etched grain seeds, disinfection, continuous flow.

### INTRODUCTION

Cereals are one of the most important groups of cultivated plants in human economic activity, giving primarily grain, which is the main food for humans and feed for farm animals (Antipov, 2017). In the world, the loss of grain from pests is enormous. Every year, when storing grain in granaries, losses from pests amount to 5-7% of the harvest. Among the pests of grain stocks, there are the most harmful and resistant species, which include: barn weevils, mites, flour beetles, barn moth, mill moth and other species (Kukharev, 2017).

As a result of the vital activity of pests, not only does the mass of products decrease, but their quality is significantly reduced. They become lumpy and unsuitable for human consumption and livestock feed. Seed germination is noticeably reduced. So, with mass reproduction of barn mites for 1-2 months, the loss of germination of wheat and rye seeds is more than 50%. Timely and effective implementation of protective measures allows you to preserve not only the yield, but also the quality of the product.

### MATERIALS AND METHODS

Grain disinfection is carried out using physical, mechanical and chemical methods. The first method includes:

- disinfection of grain by cooling, consists in maintaining a low temperature, which affects the life expectancy of grain pests;
- thermal action (drying) is often used when it is necessary to simultaneously reduce the moisture level of the grain mass. However, the use of this method is not effective when processing seed material, since it reduces the seed quality of the grain. In addition, some grain pests are resistant to high temperatures (Gorelov, 2020);
- cleaning of contaminated raw materials, which is carried out by separating them. The main disadvantage of this method is that only those pests that live in the intergranular space are removed from the grain.

Chemical methods are currently the most common methods of protecting grain storage. One of the most effective measures is seed dressing. Seed dressing is the disinfection of seed material from external and internal infection, as well as from pathogens of various bacterial and fungal diseases that spread through seeds, soil and planting material (Kukharev, 2015).

It is quite possible to disinfect cereal seeds with dressing agents such as Raxil, KS on machines that are at the disposal of Russian farms: PSSh 5, PS 10, PS 10A, Mobitox Super, sets of stationary equipment for seed plants KPS 10, K 618, K 619, as well as machines of foreign

firms, supplied as part of grain cleaning complexes such as "Petkus", "Hyde" and others (Kireev IM). However, the listed equipment does not meet modern agrotechnical requirements, therefore, for this purpose, the Department of Mechanization of Technological Processes in the Agroindustrial Complex of the Penza State Agrarian University has developed a technological scheme and a design of a device for disinfecting grain in a stream when storing it.

When performing this work, standard techniques were used using the classical provisions of theoretical mechanics, resistance of materials, theory of mechanisms and machines, mathematical modeling. (Machnev, 2016; Machneva, 2018) The development of mathematical models of the interaction of seeds with an installation for disinfecting grain in a stream during

storage and subsequent experimental studies were carried out on the basis of planning multifactor experiments and regressive analysis of experimental data with using Statistica and Matlab programs (Machnev, 2019).

The unit is designed for disinfection of grain in the flow with Prostor insect-acaricide. The installation allows processing grain with various concentrations of working solutions - from processing directly with an emulsion concentrate (15 ml of Prostor) to processing with a solution prepared in a ratio of 1 part Prostor and 4 parts of water (15 ml Prostor + 60 ml water).

The plant can be installed almost anywhere there is grain flow. This can be the head of an elevator, a dumping carriage (cart). It is also possible to mount the sprayer above the conveyor belt (Figure 1).

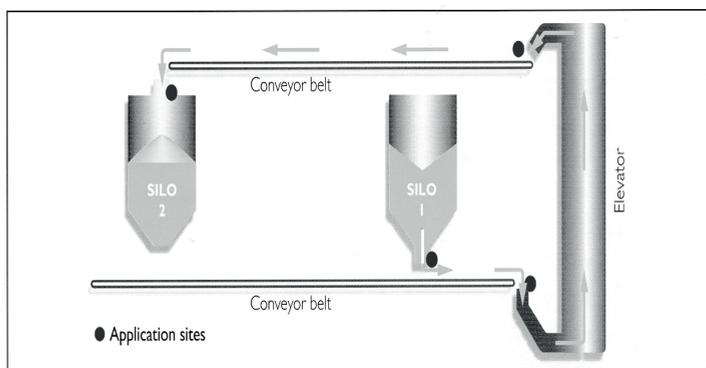


Figure 1. Installation of the proposed equipment

The unit is equipped with a fully digital control, which allows you to accurately set and control the instantaneous consumption of the drug. This makes it possible to process grain in a flow from 20 tons per hour to 500 tons per hour. In this case, two parameters are used as adjustable parameters: instantaneous flow rate and concentration of the working solution. Figure 2 shows a laboratory setup, which contains: a supporting base 1, on which a belt conveyor 4 is attached, which is driven by an electric motor 3. Above the conveyor, there is a seed hopper 5 and a ramp 7 with nozzles 8. At one end of the ramp, a pressure gauge 10 is installed for pressure control and bypass valve 9, which dump excess solution into the

pesticide reservoir 12. At the other end of the ramp, a pressure line 6 is attached.

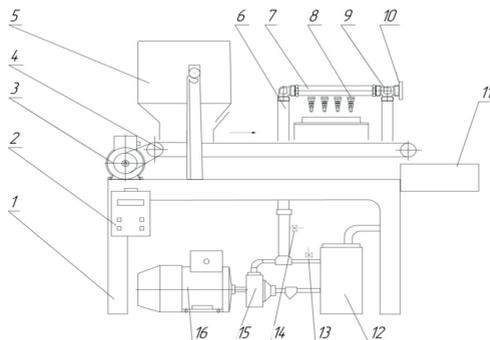


Figure 2. Installation for disinfection of grain in a stream when storing

The main operating mode is sequential. In this mode, 4 sprayers work alternately, providing a continuous flow of the preparation onto the grain.

In the event of a clogged sprayer, it is possible to turn off and redistribute the working solution flow to other sprayers.

There are currently 2 installation options:

- With control of drug consumption from a computer
- With control of drug consumption from the control controller console.

The installation also provides:

- automatic pressure regulation, which facilitates the work of maintenance personnel
- modes of checking the clogging of nozzles.

## RESULTS AND DISCUSSIONS

During the screening experiment, the three most significant factors were identified, which were fixed in the studies at the following levels of variation (Table 1).

Table 1. Factors influencing the etching process with the investigated apparatus and the levels of their variation

Designation and name of factor	Factor levels			The interval is varied
	Fundamentals	Upper	Lower	
X1 - H - Youhoneycomb mouth-Fore newbitches, mm	350	400	300	fifty
X2 - Vt - Conveyor speed, m / s	0.3	0.5	0.1	0.2
X3 - Pcr - Nozzle pressure, MPa	0.32	0.36	0.28	0.04

Three-factor experiment matrix and its results are presented in Table 2.

Table 2. Matrix and results of three factor experiment

No.	x1	x2	x3	Y, % quality seeds
one	one	one	one	90.2
2	one	one	-one	92.91
3	one	-one	one	89.49
four	one	-one	-one	87.2
five	-one	one	one	88.3
6	-one	one	-one	92.16
7	-one	-one	one	89.4
8	-one	-one	-one	91.8
9	one	0	0	93.94
ten	-one	0	0	91.25
eleven	0	one	0	93.95
12	0	-one	0	91.58
thirteen	0	0	one	92.47
14	0	0	-one	94.83
15	0	0	0	97

The experimental results were processed by the MultipleRegression module of the Statistica 6.0 software.

After processing the results of a multivariate experiment on a PC, an adequate second-order mathematical model was obtained that describes the dependence  $Y = f(H, Vt, Pav)$  in encoded form:

$$Y = 96.16089 - 0.39900x_1 + 0.80500x_2 - 0.42200x_3 - 2.10611x_1x_2 - 1.93611x_2x_3 - 1.05111x_1x_3 - 0.80750x_1x_2x_3 - 0.73000x_1x_3 - 0.89500x_2x_3 \quad (1)$$

To study the response surface, two-dimensional sections were built (Figure 3).

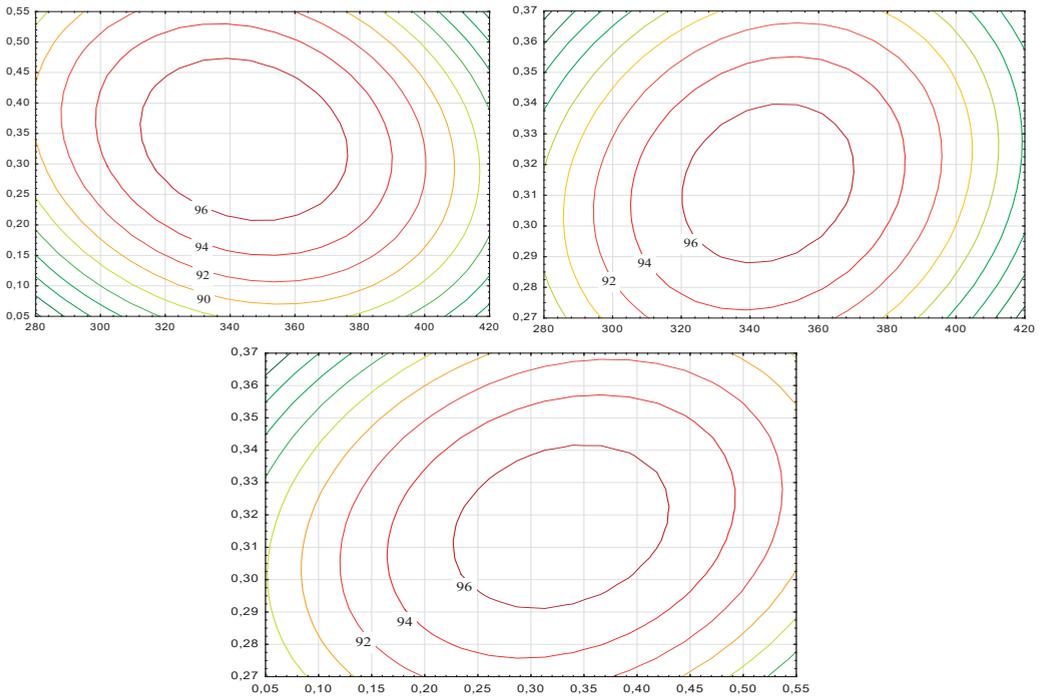


Figure 3. Two-dimensional sections of the dependence of the percentage of quality-treated seeds on the height of the nozzle installation, the speed of the conveyor and the pressure in the nozzle

Analysis of graphical images of two-dimensional sections shows that the highest value of seed dressing quality  $P = 96\%$  of the total amount of seeds can be achieved with a nozzle installation height  $H = 320 \dots 370$  mm, conveyor speed  $V_t = 0.23 \dots 0.43$  m/s, pressure in the nozzle  $P_{av} = 0.29 \dots 0.34$  MPa.

## CONCLUSIONS

There are many ways of pre-sowing seed preparation, but the purpose of all of them is the same - to increase the sowing and yield qualities of seeds. Seed dressing is an integral part of the technological process of growing crops, the need for which is beyond doubt. The proposed design of the plant for seed dressing will make it possible to achieve the value of seed dressing quality  $P = 96\%$  of the total amount of seeds. The calculations of the technical and economic assessment show that the payback period will be 0.72 years. All this speaks about the economic feasibility of using a plant for seed treatment.

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## ASSESSMENT OF MINERAL ELEMENTS CONTENT OF DIFFERENT SESAME GENOTYPES

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### Abstract

*Mineral elements play important role in plants, animals and the human body. Micronutrient deficiencies are a vital problem and affected over 3 billion people in the World. Biofortification is one of the effective and sustainable interventions to fight micronutrient deficiency. Sesame contains an average of 50% oil and 85% of this oil consists of unsaturated fatty acids. However, despite its high oil content, sesame is mostly utilized as a whole seed in the world. Therefore, it is important to determine the mineral elements contained in sesame seeds. This study was conducted to assess for 9 mineral element content of 55 sesame genotypes from 22 countries. The N, P, K, Ca, Mg, Fe, Zn, Mn and Cu contents were varied between 20.9-38.1, 2-9.9, 3.2-9.9, 8.2-24.8, 5-8.1 g kg<sup>-1</sup>, 18.9-280.8 mg kg<sup>-1</sup>, 38.2-88.9 mg kg<sup>-1</sup>, 12.5-25.7 mg kg<sup>-1</sup> and 15.3-45.7 mg kg<sup>-1</sup>, respectively.*

**Key words:** sesame, biofortification, iron, zinc, hidden hunger.

### INTRODUCTION

The human body requires around 40 known nutrients in optimum amounts to live a healthy and productive life.

These nutrients play crucial roles in human physical and mental development (White and Broadley, 2005). After the 1960's, agricultural researchers have focused on increasing agricultural productivity not quality of crops (Karaköy et al., 2012; Kurt et al., 2018). However, this has led to unforeseen negative consequences such as an increase in the prevalence of micronutrient malnutrition diseases. Today, micronutrient malnutrition is estimated to affect over half of global population. Deficiencies in Fe and Zn are the most common types of micronutrient malnutrition in the world. More than 2 billion people are affected by Fe deficiency while over 1 billion people are Zn and Se deficient (Pandey et al., 2017; Kurt et al., 2020). The primary reason for the prevalence of mineral malnutrition worldwide is the consumption of foods with very low micronutrient content.

Also, micronutrient malnutrition affects about 38% of pregnant women and 43% of pre-school children worldwide. Interventions to decrease the issue of mineral malnutrition have been implemented such as food fortification,

pharmaceutical supplementation and dietary diversification (Steina et al., 2005). However, none of these approaches have proven sustainable for various reasons such as poor delivery systems and infrastructure in developing countries. Biofortification is the process of increasing the bioavailable concentrations of vitamins and minerals in edible crops through agronomic practices, plant breeding or genetic selection to improve nutritional status (White and Broadley, 2005; Kanatti et al., 2014; Bouis and Saltzman, 2017). Fertilization is one of the remedies used to enhance mineral concentration in staple crops. Though this intervention increases leaf mineral concentrations and improves yield, it does not always enhance mineral concentration in the seed (White and Broadley, 2005). Additionally, use of fertilisers may be problematic, both economically and environmentally. Exploitation of natural biodiversity to improve the micronutrient quality of staple crops is important in breeding programs.

Sesame (*Sesamum indicum* L.) seed, the oldest oilseed crops known, is used mostly in bakery products and also made into tahini (sesame butter) and halva (sweet confections made from tahini) (Kurt et al., 2020).

Sesame seed contains oil (50%), protein (24%) and microelements (Fe, Zn, Ca, Mg, and Cu etc.).

Although sesame is generally grown in developing countries, it is consumed in varying amounts by almost every country. Hence, it is important to determine the nutrient content of sesame genotypes to provide the necessary nutritional information to the grower and consumer. Also, characterization of genetic resources has always remained one of the important methods of the breeders to investigate the novel variations which can be used for the development of improved cultivars expressing higher yield with better quality, biotic and abiotic stress resistance (Martins et al., 2006; Kurt et al., 2020).

The aim of this study was to evaluate the variability of 9 mineral elements (Fe, Zn, Cu, Mn, N, P, K, and Mg) among 55 sesame accessions from Turkey and 22 other countries.

## MATERIALS AND METHODS

*Plant Material.* A panel of fifty-five sesame accessions was used as plant material in this study (Table 1). The field experiment was conducted in an augmented design at the Experimental Farm of Cukurova University

(37.014564, 35.356717, and 23 m) in Adana, Turkey during the 2019 growing season. Before planting, 200 kg ha<sup>-1</sup> of diammonium phosphate (36 kg ha<sup>-1</sup> N, 92 kg ha<sup>-1</sup> P) fertilizer was applied, while ammonium nitrate (33% N) at the rate of 200 kg ha<sup>-1</sup> was applied before the first irrigation. Sesame seeds were sown in the second week of June. The accessions were grown in two-row plots of 5 m row length with a row spacing of 70 cm and intra-row spacing of 15 cm. Thinning was carried out after 25 days of sowing to secure one plant per 15 cm. Sprinkler irrigation was established immediately after sowing and thereafter used as needed. Weeding was carried out by hand and no herbicides were applied during the experiment. All the plants were harvested by hand in the last week of September.

*Microelement analysis.* Samples from 55 genotypes grown in 2019 were subjected to mineral element analysis using an inductively coupled plasma optical emission spectrometer (ICPOES; Vista-Pro Axial; Varian Pty Ltd., Australia). Clean sesame seed samples were taken from every population with 3 replicates and the seeds were bulked. Analysis of the microelements was performed according to the method described by Karaköy et al. in 2012.

Table 1. The list of sesame accessions

Origins	Origins	Origins	Origins
Afghanistan1	Japan 2	Taiwan	Turkey-Kütahya-Gediz
Afghanistan2	Japan 3	Turkey-Diyarbakır -Ergani-Dağarası 2	Turkey-Kütahya-Tavşanlı
China 1	Jordan	Turkey-Diyarbakır-Bismil-Bakacak	Turkey-Orhangazi-99 ©
China 2	Libya 1	Turkey-Diyarbakır-Ergani-Gisgis	Turkey-Osmaniye
Egypt	Libya 2	Turkey-Diyarbakır-Ergani-Gülbaran	Turkey-Şanlıurfa-Suruç
Greece	Mexico	Turkey-Diyarbakır-Lice-Duruköy	Turkey-Tekirdağ-Şarköy
India 1	Mozambique	Turkey-Izmir1	Turkey-Uşak-Sivaslı
India 2	Myanmar	Turkey-Izmir2	USA
Iran	Nepal	Turkey-Izmir3	Venezuela 1
Iraq	Pakistan	Turkey-Izmir4	Venezuela 2
Israel 1	Russia	Turkey-Izmir5	Zaire
Israel 2	South Korea 1	Turkey-Izmir-Tire	
Japan 1	South Korea 2	Turkey-İçel-Anamur	

## RESULTS AND DISCUSSIONS

Although sesame is one of the important oil plants, it is also a rich food source in terms of minerals such as Fe, Zn, and Mg. The nine mineral elements found in 55 sesame accessions from different countries are shown in Table 2 and Table 3. The mean values

obtained were 56.32, 66.79, 18.45, 28.08 mg/kg, 3.03, 0.50, 0.65, 1.768, 0.722% for Fe, Zn, Mn, Cu, N, P, K, Ca and Mg, respectively. The sesame accessions contained high amounts of N, P, K, Ca and Mg with mean values of 30.3, 5, 6.5, 17.68 and 7.22 g kg<sup>-1</sup>, respectively (Table 2).

Table 2. Macro element content of sesame accessions

Origins	Seed color	N g kg <sup>-1</sup>	P g kg <sup>-1</sup>	K g kg <sup>-1</sup>	Ca g kg <sup>-1</sup>	Mg g kg <sup>-1</sup>
Afghanistan 1	BLK	30.2	4.3	6.1	22.3	7.5
Afghanistan 2	DB	26.2	6.5	6.5	23.4	7.4
China 1	LB	31.0	3.9	7.1	18.1	7.6
China 2	G	31.8	3.5	4.9	20.7	6.4
Egypt	W	34.0	3.8	6.6	12.8	7.2
Greece	BLK	33.2	5.3	6.9	27	7.6
India 1	BLK	31.9	5.3	6.1	20	7
India 2	W	30.5	2	6.6	24.8	7.7
Iran	Y	30.4	3	5.9	18.5	5
Iraq	BLK	29.5	3.6	5.6	22.6	7.5
Israel 1	DB	32.4	4.6	5.7	20.8	7.7
Israel 2	BLK	38.1	6.7	6.4	23.6	7
Japan 1	BLK	35.3	4.6	5.8	18.6	7.4
Japan 2	DB	31.2	3.2	6	24.4	7.5
Japan 3	DW	31.8	3.4	6.5	16.4	7.8
Jordan	W	29.8	9.9	4.5	8.2	5.3
Libya 1	B	29.8	3.9	7.1	21.4	7.6
Libya 2	BLK	32.7	9.8	6.4	10.3	6.4
Mexico	B	32.1	3.7	6.6	24.4	7.4
Mozambique	W	30.2	4.6	7.2	18	7.6
Myanmar	DG	30.2	3.5	5.2	27	6.8
Nepal	DB	26.9	4.5	3.2	15.6	5.5
Pakistan	W	31.0	4.5	7.2	21.3	7.2
Russia	DB	29.9	6.2	7.7	21.2	7.5
South Korea 1	DW	31.8	3.8	7.5	14.9	8
South Korea 2	BLK	32.8	2.8	6.1	18.9	7.5
Taiwan	BLK	34.4	3.1	5.6	21.7	7.3
Turkey- Diyarbakir -Ergani-Dagarasi 2	B	27.6	3.9	7	23.9	7.8
Turkey- Sanliurfa -Siverek-Yuvakoy	LB	27.2	8.7	6.1	20.1	6.9
Turkey-Balikesir-Bandirma	W	27.1	9.1	4.3	9.1	8.1
Turkey-Bursa-Orhangazi	LB	32.7	4.9	7.8	10.3	7.7
Turkey-Denizli-Acipayam	LB	31.1	5.7	6.1	16.2	7.7
Turkey-Diyarbakir -Ergani-Dagarasi 1	LB	32.8	2.9	5.7	18.9	7.8
Turkey-Diyarbakir-Bismil-Bakacak	BLK	25.5	3.3	6.3	17.5	6.7
Turkey-Diyarbakir-Ergani-Gisgis	B	29.3	4.6	5.7	16.1	6.3
Turkey-Diyarbakir-Ergani-Gülbaran	DB	27.9	1.5	5.7	18.1	6.8
Turkey-Diyarbakir-Lice-Durukoy	DB	28.6	4.1	6.1	16.6	7
Turkey-Izmir 1	B	28.3	4.5	9	16	7.5
Turkey-Izmir 2	DB	26.7	4.9	5.7	21	7.3
Turkey-Izmir 3	DB	25.7	8.5	9.9	13.3	7.4
Turkey-Izmir 4	W	28.5	3.8	4.4	10.4	5.6
Turkey-Izmir 5	Y	34.6	5.3	6.7	21.6	8
Turkey-Izmir-Tire	LB	25.8	3.4	7.1	16.2	7.6
Turkey-İcel-Anamur	LB	26.2	8.5	5.7	16.8	7.6
Turkey-Kutahya-Gediz	BLK	29.8	5.2	6.4	12.1	7
Turkey-Kutahya-Tavsanli	W	24.5	3.7	6.8	10.3	6.6
Turkey-Orhangazi-99 ©	B	35.0	7.1	9.2	16.3	7.9
Turkey-Osmaniye	B	31.3	5.4	5.5	14.1	7.3
Turkey-Sanliurfa-Suruc	LB	29.6	3.6	7.7	9.8	6.9
Turkey-Tekirdağ-Sarkoy	B	20.9	5.4	6.1	11.8	7.3
Turkey-Usak-Sivasli	Y	27.8	9.1	6.2	13.2	7
USA	B	31.1	7.7	7.3	17.2	8.1
Venezuela 1	DW	34.1	8.9	8.9	19.2	8
Venezuela 2	W	33.8	4.3	7.5	20.9	8
Zaire	W	33.8	5.7	7	8.7	7
<b>Average</b>		<b>30.3</b>	<b>5.0</b>	<b>6.5</b>	<b>17.68</b>	<b>7.22</b>
<b>Min.</b>		<b>20.9</b>	<b>2.0</b>	<b>3.2</b>	<b>8.2</b>	<b>5.0</b>
<b>Max.</b>		<b>38.1</b>	<b>9.9</b>	<b>9.9</b>	<b>24.8</b>	<b>8.1</b>

©: Turkish cultivar; B: Brown; DB: Dark Brown; LB: Light Brown; BLK: Black; Y: Yellow; W: White; DW: Dirty White; G: Grey

Table 3. Micro elements content of sesame accessions

Origins	Seed color	Fe (mg/kg)	Zn (mg/kg)	Mn (mg/kg)	Cu (mg/kg)
Afghanistan 1	BLK	73.2	67.3	19.7	40.2
Afghanistan 2	DB	51	66.7	17.5	18.9
China 1	LB	51	61.1	16.1	24.8
China 2	G	91.2	50.3	15.4	32.8
Egypt	W	66	60.6	12.8	40.4
Greece	BLK	59.4	68.4	19.7	30.1
India 1	BLK	53.1	48.7	17.1	37.2
India 2	W	46.8	67.5	13.7	28.6
Iran	Y	60	66	13.6	33.2
Iraq	BLK	43.2	71.9	22	24.6
Israel 1	DB	61.5	65.1	14.9	16.6
Israel 2	BLK	71.4	80.4	23.2	23.3
Japan 1	BLK	77.4	64	16.4	37.3
Japan 2	DB	43.2	69.5	17.1	18
Japan 3	DW	63.3	73	17	37.2
Jordan	W	40.6	45.3	14	15.5
Libya 1	B	55.5	78.7	17.9	23.1
Libya 2	BLK	27.6	58.7	17.1	20.7
Mexico	B	41.1	64.6	17.8	30.8
Mozambique	W	34.2	69.6	<b>12.5</b>	15.7
Myanmar	DG	61.8	71.9	20.2	26.1
Nepal	DB	48	47.4	11.7	23.1
Pakistan	W	27.3	52.5	22.1	25.4
Russia	DB	75	50.4	20.6	40.6
South Korea 1	DW	31.8	79.1	19.9	27.5
South Korea 2	BLK	<b>280.8</b>	80.2	23.0	32.1
Taiwan	BLK	75.3	79.7	16.1	39
Turkey- Diyarbakir -Ergani-Dagarasi 2	B	48	79.7	20.4	26.7
Turkey- Sanliurfa -Siverek-Yuvakoy	LB	36.6	72.8	23.1	31.6
Turkey-Balikesir-Bandirma	W	25.7	<b>38.2</b>	14.6	15.3
Turkey-Bursa-Orhangazi	LB	26.1	74	19.4	24.6
Turkey-Denizli-Acipayam	LB	33.6	68.6	15.4	22.3
Turkey-Diyarbakir -Ergani-Dagarasi 1	LB	43.8	71.9	16	31.1
Turkey-Diyarbakir-Bismil-Bakacak	BLK	31.2	64.6	22.2	27.7
Turkey-Diyarbakir-Ergani-Gisgis	B	<b>178.5</b>	65.4	<b>25.7</b>	32.7
Turkey-Diyarbakir-Ergani-Gülbaran	DB	24.6	58.4	22	19.7
Turkey-Diyarbakir-Lice-Durukoy	DB	48.3	75.7	20.9	23.3
Turkey-Izmir 1	B	61.8	76.6	25.2	39.3
Turkey-Izmir 2	DB	77.7	68.3	22	37.4
Turkey-Izmir 3	DB	78	74.7	22.1	36.9
Turkey-Izmir 4	W	68.7	70.1	20.9	<b>45.7</b>
Turkey-Izmir 5	Y	55.8	74.3	12.8	12.9
Turkey-Izmir-Tire	LB	34.8	60.1	22.6	29
Turkey-İcel-Anamur	LB	33.3	55.5	14.5	27.1
Turkey-Kütahya-Gediz	BLK	30.6	65.5	19.4	28.5
Turkey-Kütahya-Tavsanli	W	<b>18.9</b>	73.5	21.7	25.4
Turkey-Orhangazi-99 ©	B	34.8	<b>88.9</b>	23.7	25.7
Turkey-Osmaniye	B	56.4	64.8	16.4	35.3
Turkey-Sanlıurfa-Suruc	LB	31.8	59.3	23.7	32.7
Turkey-Tekirdağ-Sarkoy	B	49.8	66.5	16.6	29.3
Turkey-Uşak-Sivasli	Y	36	59.6	15	19.2
USA	B	72	76.6	20.2	30.5
Venezuela 1	DW	60	62.4	17.4	28.2
Venezuela 2	W	42	83.8	13.5	<b>12.9</b>
Zaire	W	48	65	18.5	30.6
<b>Average</b>		<b>56.32</b>	<b>66.79</b>	<b>18.45</b>	<b>28.08</b>
<b>Min.</b>		<b>18.90</b>	<b>38.20</b>	<b>12.50</b>	<b>12.9</b>
<b>Max.</b>		<b>280.8</b>	<b>88.9</b>	<b>25.7</b>	<b>45.7</b>

©: Turkish cultivar; B: Brown; DB: Dark Brown; LB: Light Brown; BLK: Black; Y: Yellow; W: White; DW: Dirty White; G: Grey

These values were higher than reported in previous studies (Deme et al., 2017; Wacal et al., 2019; Özcan, 2006). The Recommended Daily Intakes (RDIs) of Mg for adult men is 350 mg, while for women this value is 300 mg. For children aged 3 to < 10 years, an RDI for magnesium is set at 230 mg/day for both sexes (EFSA, 2015). The results show that sesame seeds are an alternative food source that will contribute to meet the daily Mg need for children.

The Mn values of the 55 sesame accessions varied between 12.5-25.7 mg kg<sup>-1</sup> and the average being 18.45 mg kg<sup>-1</sup>. Turkey-Diyarbakır-Ergani-Gisgis accession had the highest Mn (25.7 mg kg<sup>-1</sup>) and the lowest was for Mozambique (12.5 mg kg<sup>-1</sup>). The Cu values ranged from 12.9 (Venezuela 2) to 45.7 (Turkey-Izmir 4) mg kg<sup>-1</sup> is the average value 28.08 mg kg<sup>-1</sup>. The Cu content in this study was higher than that reported by Hu and Zhou (2019), Wacal et al. (2019), Kurt et al. (2018) and Pandey et al. (2017). Zinc is one of the most important micronutrients in biological systems and plays a vital role in protein synthesis and metabolism. Several Zn-binding proteins are transcription factors necessary for gene regulation and necessary for more than half of enzymes and proteins involved in ion transport (Andreini et al., 2006). Any decrease in Zn concentration in the human body may result in a number of cellular dysfunctions, including a high susceptibility to infectious diseases, retardation of mental development, and stunted growth of children (Black, 2003). The highest Zn value was obtained from the Turkey-Orhangazi-99 cultivar (88.9 mg kg<sup>-1</sup>), while the lowest value was obtained from Turkey-Balikesir-Bandirma accession (38.2 mg kg<sup>-1</sup>). The most of Turkish accessions had Zn content higher than the mean value.

The RDIs of Zn for adult men is 11 mg, while for women this value is 8 mg. This value is 5 mg/day for children <8 years old. Due to its high zinc content, sesame has the potential to be an important food source in fighting zinc deficiency, especially in children. In fact, if approximately 30 grams of sesame seeds are eaten daily from Orhangazi-99 variety, half of the daily need will be met. Although iron is very important for human health, it is the most common micro element deficiency. The most

important reason for this is feeding with cereals with low iron content. Fe content in sesame accessions of different origins ranges from 18.9 (Turkey-Kütahya-Tavsanlı) to 280.8 (South Korea 2).

Among the Turkish accessions, the highest Fe content belonged to the Turkey-Diyarbakır-Ergani-Gisgis accession (178.5 mg kg<sup>-1</sup>), while the Turkey-Kütahya-Tavsanlı (18.9 mg kg<sup>-1</sup>) accession had the lowest value. In the present study, the average Fe content value was lower than reported in earlier studies (Hu and Zhou, 2019; Wacal et al., 2019; Kurt et al., 2018; Obiajunwa et al., 2005), while higher than Deme et al. (2017). It has been determined that there is a significant variation between sesame accessions in terms of the traits examined.

## CONCLUSIONS

In this study, significant variation was found in all studied mineral elements among sesame accessions from different origins.

The results show that sesame seeds contain rich essential mineral elements important for the human body and can play an important role in fighting malnutrition.

Sesame breeders can use the results of the present study to further the breeding programs for the development of new biofortified varieties.

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## ASSESSMENT OF ANTIFUNGAL ACTIVITY OF GOLD-CHITOSAN AND CARBON NANOPARTICLES AGAINST *Rhizoctonia solani* Kühn

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### Abstract

Nanoparticles are implemented in different biotechnological fields, and there is interest in their use in plant biology. Nanotechnology can help overcome the persistent limitations of using conventional fungicides in the management of plant diseases, contributing to a safer environment. Hence, this study is focused on evaluating the behavior of nanoparticles on *Rhizoctonia solani*, which has a worldwide distribution and causing important economic losses to many agricultural and horticultural crop species. *Rhizoctonia solani* (teleomorph: *Thanatephorus cucumeris*) is an basidiomycetous fungus that is well-known as a soil borne plant pathogen, adapted to any soil type, and it lives in different forms on plant debris. Gold-chitosan and carbon nanoparticles were suspended in malt extract peptone agar nutritive media, and their antifungal activity was evaluated at 24, 48, 72 and 96 hours after incubation by measuring the diameter of fungal colonies. The results showed that gold-chitosan nanoparticles have antifungal properties against *R. solani*, the fungal colony growth diameter being reduced. Likewise, it was observed that the colony diameter was smaller when the nanoparticle concentration increased. However, the highest carbon nanoparticle concentration applied during the experiment's execution was not able to inhibit *R. solani* growth.

**Key words:** gold-chitosan nanoparticles, carbon nanoparticles, antifungal activity, *Rhizoctonia solani*.

### INTRODUCTION

Temperature and humidity play important roles in plant biology during the growth of plants in the field. Filamentous fungi can harm and degrade plants at any time during their phenophases or after harvesting, reducing the germination potential of seeds or the nutritional value of plant products (Sturrock et al., 2015).

*Rhizoctonia solani* (teleomorph: *Thanatephorus cucumeris*) is one of the most destructive pathogens infecting a wide range of crops around the world and causes a wide range of plant diseases (e.g. black scurf of potato, bare patch of cereals, tomato foot and root rot, root rot of sugar beet, belly rot of cucumber, damping off in soybean seedling) (Akgun et al., 2018; Nikraftar et al., 2013). The pathogen is not efficiently controlled by resistance breeding and is necessary to plan other effective disease control strategies in crop protection beside conventional fungicides. One of the strategies

is the usage of new antifungal substances in order to control and inhibit fungal growth.

Antifungal materials, which may also serve as biostimulants, allowing plants to expand (through different processes) when used in limited amounts, are an interesting group of materials. Nanoparticles (NPs) are a type of biostimulant that have a high density of surface charges that interact with the surface charges found on cell walls and membranes (Juárez-Maldonado et al., 2019). Furthermore, because of the various possibilities for modifying the combination of their physical and chemical properties, NPs have begun to be used as new forms of antimicrobial agents. The antimicrobial activity of NPs is related to their association with functional groups on the surface of microorganism cells, which results in the inactivation of the microorganism (Cui et al., 2012). Just a few reports on the effects of gold or carbon nanoparticles on bacteria (Perni et al., 2009) or fungi (Ahmad et al., 2013; Lipsa et al.,

2020) have been published. Chemical reduction, sol-gel processes, gas condensation, electrodeposition or vacuum deposition and vaporization, and pulsed laser ablation in liquid (PLAL) can all be used to create NPs. To extract NPs from a bulk solution, a reducing agent (e.g., sodium borohydride, ascorbic acid, sodium citrate, amino acids) is used. PLAL is a flexible technique for making surfactant-free stable colloidal solutions of nanoparticles from a variety of materials (Zeng et al., 2012). Gold nanoparticles were synthesized using chitosan (AuNPs–chitosan), which acts as both a reducing and stabilizing agent, and carbon nanoparticles (CNPs) were synthesized using PLAL. Due to their inert nature, gold NPs are considered to be nontoxic, unlike other inorganic nanoparticles (Lipsa et al., 2020; Rahimi et al., 2019).

Carbon nanomaterial quality assurance research is gaining traction (Ursu et al., 2019; 2020), with the aim of facilitating their use in a variety of applications (Chung et al., 2011), including plant science, drug delivery systems, energy storage devices, bioimaging, and biosensors. Carbon nanomaterials come in a variety of forms (e.g., carbon nanotubes, carbon nanoparticles, and carbon dots), and although they provide a number of benefits, their possible toxicity to the environment is a significant consideration (Poland et al., 2008). However, depending on the experimental procedure used to create them, certain carbon nanomaterials can be toxic while others are not (Firme et al., 2010). As a result, researchers are looking for new ways to make nontoxic carbon nanoparticles with the aim of using them safely to boost crop production (Zaytseva & Neumann, 2016). The purpose of this study is to evaluate the fungicidal activity of AuNPs-chitosan and CNPs on *Rhizoctonia solani*.

## MATERIALS AND METHODS

### *Synthesis of Chitosan-Stabilized Gold Nanoparticles*

Solutions of tetrachloroauric acid (HAuCl<sub>4</sub>; 0.01 M) and 0.1 mg/mL chitosan were combined in different ratios and then treated with an ultrasonic field (for 20 minutes at a temperature of 55°C to produce AuNPs-chitosan with concentrations of 25, 50, and

75 g/mL. The gold concentration was used to mark the samples. AuNP25, for example, is the name given to a sample of AuNPs-chitosan with a concentration of 25 g/mL.

### *Synthesis of Carbon Nanoparticles*

The PLAL method was used to create stable CNP suspensions in ethanol that were free of surfactants. As a result, two CNP solutions for two laser fluences of 2 and 3 J/cm were obtained, resulting in samples labeled CNP 1 and CNP 2, respectively. CNP 1 had a concentration of 19 mg/mL, while CNP 2 had a concentration of 23 mg/mL.

### *Nanoparticles Application to Fungi*

The Leibniz Institute German Collection of Microorganisms and Cell Cultures (Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) provided the *Rhizoctonia solani* DSM 22844 strain used in this research. The phytopathogenic fungi were routinely grown at 28°C in the climatic chamber on malt extract-peptone-agar (MEPA) medium containing 30 g malt extract, 3 g soya peptone and 15 g agar (Merck, Germany).

The antimicrobial properties of AuNPs-chitosan and CNPs against *R. solani* strain were studied at different concentrations, ranging from 25 to 75 g/mL for AuNPs-chitosan and 19 to 23 mg/mL for CNPs.

The gold and carbon-based NPs were suspended in 15 mL MEPA medium and poured into Petri dishes with a diameter of 90 mm. The NP solutions were applied to Petri dishes in various doses (0, 0.5, 1, 2, and 5 mL) in order to determine the most effective dose for each NP solution (regardless of the initial concentration of the respective solution). Using a media preparator with automatic dispenser (Masterclave 09 + APS 320/90 AES Laboratoire, France) for Petri plates, the MEPA medium was prepared and poured.

After cooling and solidification, the plates were inoculated aseptically with 7 mm diameter disks of *R. solani* taken from an actively developing edge of a five-day-old culture and incubated at 28°C for 4 days, supplemented with different concentrations of NPs.

Every 24 hours, the fungal plaque diameter from the inoculated plates was determined, and the mycelial development was photographed. For each microbial determination, the

procedure was carried out three times. To measure the percentage of growth inhibition, the obtained values were compared to those of the control (without NPs) using Formula (1):

$$\% \text{ Inhibition rate} = \frac{(M_c - M_t)}{M_c} \times 100, \quad (1)$$

where  $M_c$  is the mycelial growth for the control plate, and  $M_t$  is the mycelial growth for the plates treated with different dosage of NP solutions. Values are shown in terms of mean and standard deviation (mm).

#### Statistical Analysis

A two-sample t-test with unequal variances was used to assess the effect of nanoparticles on *R.*

*solani* strain using Microsoft Excel 2016 software (Microsoft, USA). The differences were considered statistically important at the 0.05 likelihood level ( $p < 0.05$ ) when the values were compared to the average of all the values in the experiment.

## RESULTS AND DISCUSSIONS

The aim of this study was to determine the inhibitory action of AuNPs-chitosan and CNPs on colony formation from the mycelia of *R. solani* under laboratory conditions (*in vitro*). Figure 1 shows the growth rate of *R. solani* strain in the presence of the measured NPs.

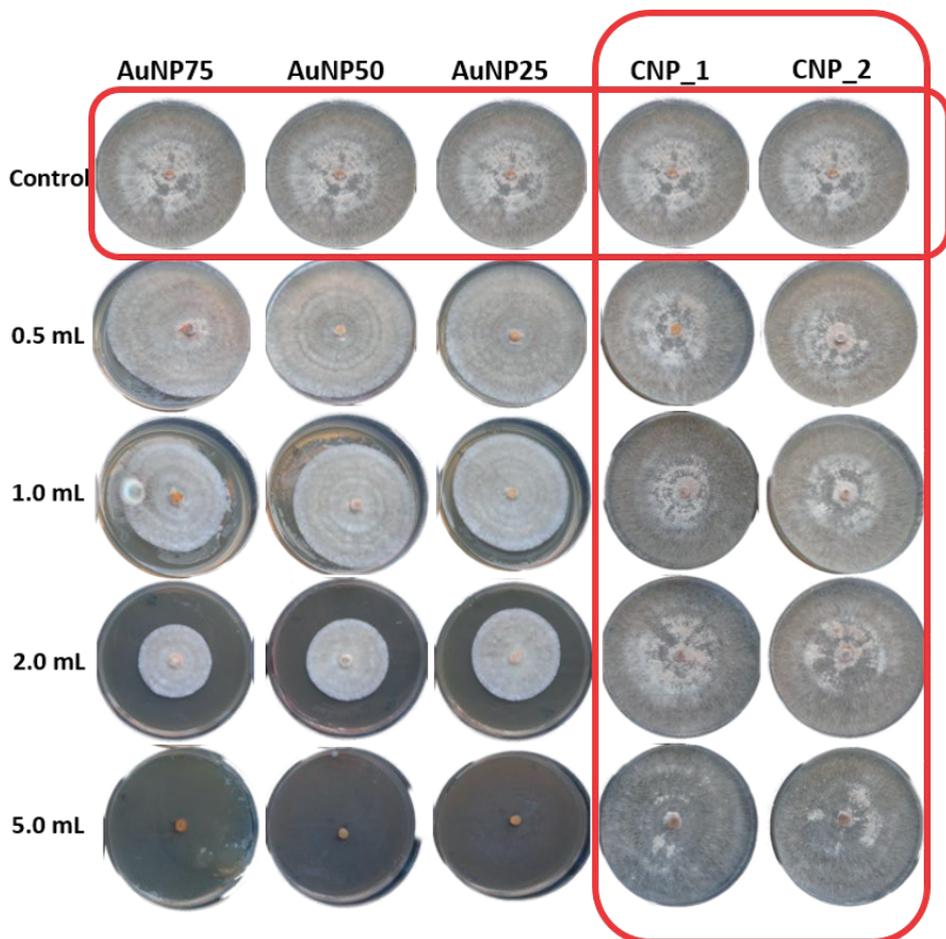


Figure 1. Effects of the interactions of different nanoparticles at different concentrations and doses on the inhibition of mycelial growth of *Rhizoctonia solani* DSM 22844 strain. There was no inhibition in the case of the control plates. For all the controls, the same picture was used as there was no difference between the control plates.

The tested NPs inhibited radial growth of *Rhizoctonia solani* strain in different ways at different concentration levels. When compared to the lowest dosage (0.5 mL), AuNPs-chitosan exhibited inhibitory effects at the maximum dosage (5 mL). Figures 1 and 2 show the

impact of AuNPs-chitosan and CNP concentrations on the mycelial growth of *R. solani* strain tested after 4 days of incubation. In case of CNPs no inhibitory effects were observed and no information are present in Figure 2.

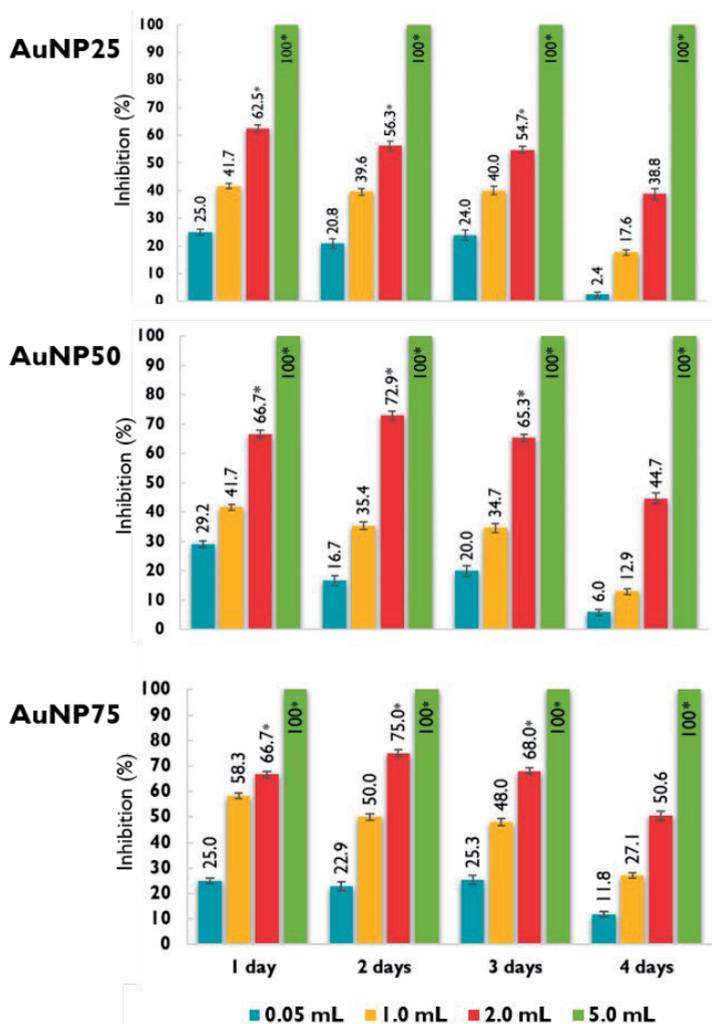


Figure 2. Antifungal activity of gold-chitosan (AuNPs-chitosan) nanoparticles applied at different concentrations and dosage on *Rhizoctonia solani* DSM 22844 strain on MEPA medium after 1, 2, 3, and 4 days of incubation at 28°C. *Rhizoctonia solani* DSM 22844 strain with different concentrations, ranging from 25 to 75 µg/mL in the case of AuNPs-chitosan. The percentages of growth inhibition were calculated in relation to the control. Error bars represent a standard deviation from the mean ( $n = 3$ ). Significant differences ( $p < 0.05$ ) are marked with an asterisk.

The application of AuNPs-chitosan at different concentrations and dosage was effective on *R. solani* strain; in the case of the pathogen, a mycelial growth inhibition rate of 100% ( $p < 0.05$ ) was observed. Absolute inhibitions were obtained through the application of 5.0 mL

AuNPs-chitosan for all three different concentrations (i.e. AuNP25, AuNP50, and AuNP75), the measurements being made after 1, 2, 3, and 4 days, respectively. Furthermore, a decrease in mycelial growth inhibition was observed throughout the four-day trial period

when AuNPs-chitosan concentrations and dosages were lower. In the case of the *R. solani* DSM 22844 strain, the results shown in Figure 2 revealed that AuNP25 had no antifungal activity at a dosage of 0.5 mL. In almost all cases, the efficacy of all gold-chitosan nanoparticles is observed to increase with dosage and concentration. The antifungal activity of AuNPs-chitosan against the plant pathogen could be due to a synergistic effect between gold nanoparticles and chitosan.

Antifungal activity of gold nanoparticles against plasma membrane proteins is size dependent (Ahmad et al., 2013). NPs can cause activity loss by interacting directly with enzymes involved in the regulation of the proton gradient across the plasma membrane. The fungal membrane will then be unable to regulate H<sup>+</sup> transport, resulting in cell growth retardation and death (Beyenbach & Wieczorek., 2006). AuNPs can diffuse through the cell membrane and interact with sulfur-containing proteins in the membrane or phosphorus-containing bases in the cells' DNA to inhibit synthesis, reparation, and replication, leading to cell death, according to another study (Tan et al., 2011).

Chitosan is among the most promising plant pathogen-fighting compounds. It may increase antimicrobial activity by binding to the cell surface and inhibiting pathogen growth alone or in combination with other nanoparticles. Chitosan's antimicrobial activity is affected by its form, molecular weight, and concentration. Chitosan with a low molecular weight can pass through the fungal membrane and inhibit microbial growth. Chitosan attaches to the cell surface of microorganisms at low concentrations, causing membrane disruptions and ultimately the death of the microbial cell due to leakage of intracellular components (Hosseinnejad & Jafari, 2016).

Another part of our study consists of using CNPs at different concentrations (CNP\_1 = 19 mg/mL, CNP\_2 = 23 mg/mL) and doses (0.5, 1, 2, and 5 mL) to analyze the inhibitory effects on *R. solani* DSM 22844 strain. From Figure 1, it can be observed that 4 days after inoculation and at the highest dosage (5 mL per Petri dish), no inhibition in the case of the DSM 22844 strain was attained.

In the case of both CNPs (CNP\_1 and CNP\_2), despite the fact that the dimension of CNPs were different (CNP\_2 = 97 nm vs. CNP\_1 = 120 nm in diameter), the results showed no influence on the mycelia growth, regardless of the exposure doses, on *R. solani* DSM 22844 strain (data and figure not presented).

No inhibition on growth rates was registered because the effects of CNPs within different biological systems (plants, microorganisms) are diverse and dependent on the CNP type, its physical characteristics, type of organism, dosage/concentration, method of application, and duration of the exposure. In plants, Verma et al. (2019) found that CNPs were successful at enhancing water uptake and transport, seed germination, activating water channel proteins, and promoting nutrient absorption at lower concentrations. All of these improvements were absent when the CNP concentration was high.

In *R. solani* strain, statistically significant ( $p < 0.05$ ) mycelial growth inhibition was observed using the findings presented above for AuNPs-chitosan. Significant differences were found for 2-mL and 5-mL NP dosage (Figure 2).

## CONCLUSIONS

In agricultural fields, the use of AuNPs-chitosan is a promising alternative to the typical use of conventional fungicides to combat plant pathogens like *Rhizoctonia solani*. The current study shows that AuNPs-chitosan has antifungal activity against *R. solani*, one of the most destructive pathogens infecting a wide range of crops around the world.

The application of 5 mL of AuNPs-chitosan solution to the *Rhizoctonia solani* DSM 22844 strain resulted in absolute inhibition ( $p < 0.05$ ) for all concentrations tested (25, 50, and 75 g/mL). Also, a dosage of 2 mL AuNPs-chitosan solution presented a statistically significant differences in the first 3 days of this study. Our findings show that the dosage used when evaluating the antifungal efficacy of an NP solution is an important factor to consider (regardless of the concentration used). As a result, instead of using a high-concentration NP solution (which means higher manufacturing costs), a lower-concentration solution can be used if the correct dose is known.

To summarize, particle size, molecular weight, concentration, and dosage are essential factors that should be considered in the future developing of new fungicide formulations with applications in plant disease management. Therefore, more investigations on field applications (*in vivo*) are needed.

## ACKNOWLEDGEMENTS

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## COMPARATIVE PERFORMANCES OF ORGANIC FERTILIZERS ON DIFFERENT CROPS IN CLIMATIC CONDITIONS OF ARGEȘ COUNTY

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### Abstract

*The aim of the research was to investigate the efficiency of two fertilizers accepted for organic agriculture (CODAMIX - F1 and ECOAMINOALGA - F2) on different field crops and to compare their effects on yield performances. The experimental study developed during 2019-2020 in Albota, Argeș County is based on investigation of the variability of yield components for wheat, sunflower, maize and soybean crops under influence of organic fertilizers application. The experimental scheme was composed from four plots and three variants (control, F1 and F2) for each crop. Organic fertilizers (F1, F2) were applied foliar during vegetation period as one treatment for wheat and two treatments for the other field crops. For wheat (Trivale variety), all investigated yield parameters increased after foliar fertilization, a significant increase of total biomass and spikes biomass being noticed after F2 treatment. As concerning the sunflower (Puntasol CL hybrid) and maize (F376 hybrid) crops, the results indicated significant and distinct significant differences between treatments and control variant. In the case of soybean (Florina F variety) crop, application of organic fertilizers influenced positively all yield parameters but considering drought conditions of the year the yield was generally affected.*

**Key words:** crop, foliar application, organic fertilizer, yield component.

### INTRODUCTION

Nowadays, organic farming is equally recognized by scientists and consumers as well, as a suitable manner to generate healthy food products by avoiding chemical fertilizers and pesticides and to contribute therefore to decrease of environmental pollution. The organic production regulations allow the use of specific inputs that has to comply strict standards, the accepted products being regulated by Commission Regulation (EC) No 889/2008 and by Regulation (EC) No 2003/2003 of the European Parliament and of the Council.

Even if organic fertilizers may contribute to higher crop yield without depleting soil quality (Cen et al., 2020), many farmers are concerned that crop yield may decrease in the early stages of transition from conventional to organic systems (Tu et al., 2006). Nevertheless, some

studies evidenced that application of compost and liquid manure in organic deficient soils showed better yield performances for maize crop than those obtained under conventional farming practices (combined application of manure and mineral fertilizers) (Onduru et al., 2002).

In organic agriculture are used different traditional inputs, as compost (Erhart & Hartl, 2010), green (Carr et al., 2020) and animal manure (Adesoye et al., 2014; Carr et al., 2020; Krauss et al., 2020), crop straw (Wang et al., 2018), sewage sludge (Hammad et al., 2011) and their efficiency has been investigated. Some researchers (Cen et al., 2020) reported the efficiency of three organic fertilizers (rapeseed meal, soybean meal and cattle manure) on crop yield for a winter wheat-summer maize rotation system. Accordingly, winter wheat and summer maize presented yield increases of 161%, 299% and 256% after

rapeseed meal, soybean meal and cattle manure treatment, respectively in comparison with control variant.

Other researchers (Hammad et al., 2011) investigated the efficiency of organic manures on a spring wheat cultivar. Hence, application of different combinations of organic manures (green manure - GM, farm yard manure - FYM, poultry litter - PL, press mud - PM, sewage sludge - SS) at a rate of 10 t/ha evidenced that variant consisting from GM+PL+SS treatment produced maximum yield that was 137% higher than control variant with no fertilization. Recently, the objectives of scientists are to obtain new materials, environmental friendly, accepted for organic agriculture, which may contribute to yield increase and improvement of soil characteristics. Thus, it has been obtained compounds and mixtures that can be used to synthesize new effective fertilizer formulas. Among them, it could be mentioned protein hydrolysates of animal and vegetal origin (Mihalache et al., 2014; Mihalache & Stanescu, 2017), products that contain humic

acids (Russo & Berlyn, 1991; Ekin, 2019) and seaweed extracts (Russo & Berlyn, 1991) or microgranule fertilizers fortified with proteins (Olbrycht et al., 2020).

Having in view the importance of organic agriculture from both environmental and consumers' perspectives, it was designed an experiment with the purpose to investigate the efficiency of two fertilizers accepted for organic agriculture (CODAMIX - F1 and ECOAMINOALGA - F2) on different field crops (autumn wheat, sunflower, maize, soybean) and to compare their effects on yield performances.

## MATERIALS AND METHODS

### *Experiment location*

The experimental study was developed during 2019-2020 in Albota, Argeş County, where the dominant soil type is albic luvisols (Mihalache et al., 2015). Albota is located in the south part of Argeş County, at 10 km far from Piteşti (Figure 1).

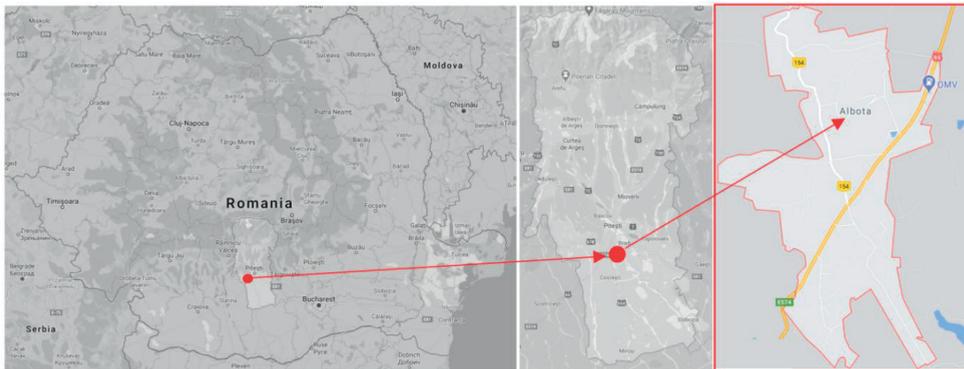


Figure 1. Position of Albota on geographical map

### *Experiment description*

To achieve the proposed objectives, it was chosen for testing the following species:

autumn wheat, sunflower, maize and soybean. The details of the experiment are presented in Table 1.

Table 1. Experiment characterization

Field crop	Autumn wheat	Sunflower	Maize	Soybean
Variety/hybrid	Trivale	Puntasol CL	F 376	Florina F
Sowing date	10.10.2019	17.03.2020	08.04.2020	13.04.2020
Lot surface*	500 m <sup>2</sup>	600 m <sup>2</sup>	600 m <sup>2</sup>	500 m <sup>2</sup>

\*3 variants/lot and 3 repetitions/variant

### Soil agrochemical characterization

The experiment was developed on albic luvisol with clay texture. Soil reaction (pH = 5.30) is moderately acidic, meanwhile humus content is considered as low level, even if during years, due to different cultural practices has increased to 2.41%. Mobile phosphorus content ( $P_{AL}$  =

39 mg/kg) corresponds to good provision level, mobile potassium ( $K_{AL}$  = 83 mg/kg) is associated with medium content, meanwhile inorganic sulphur level is 23 mg/kg and corresponds to high content.

Microelements' contents are depicted in Table 2.

Table 2. Soil microelements' content

Microelement	Total form, mg/kg	Mobile form, mg/kg
Co	9	-
Cu	14	2.8
Mn	618	51
Ni	25	-
Zn	51	1.5

### Characterization of used fertilizers

To fulfil the objectives, for study was chosen two inputs accepted for organic agriculture: CODAMIX (coded F1) and

ECOAMINOALGA (coded F2). The inputs' full chemical characterization is presented in Table 3.

Table 3. Foliar organic fertilizers characterization

CODAMIX (F1)			ECOAMINOALGA (F2)	
Guaranteed analysis	%w/w	%w/v	Parameter	Content, %
Iron (Fe) complexed and water soluble	4.00	5.12	Organic nitrogen	3
Manganese (Mn) complexed and water soluble	2.00	2.56	Organic matter	46
Zinc (Zn) complexed and water soluble	0.50	0.64	Potassium ( $K_2O$ )	6
Copper (Cu) complexed and water soluble	0.12	0.15		
Boron (B) water soluble	0.30	0.38		
Molibdenum (Mo) water soluble	0.08	0.10		
Complexing agent: lignosulfonates				

### Fertilization scheme

In Table 4 are depicted treatments for each experimental crop. The foliar treatments with

F1 and F2 inputs were applied twice, excepting autumn wheat crop, in the case of which was applied one treatment only.

Table 4. Fertilization scheme

Experimental crop	Autumn wheat	Sunflower	Maize	Soybean
Preceding crop	Sunflower	Wheat	Maize	Wheat
Basal application	Bio Enne* 250 kg/ha	Bio Enne* 250 kg/ha	Bio Enne* 250 kg/ha	$N_{30}P_{30}$ + $CaCO_3$ 1.5 t/ha
First treatment application (phenophase) <sup>#</sup>	25.05.2020 (grain filling)	25.05.2020 (6-7 leaves)	25.05.2020 (7-8 leaves)	25.05.2020 (3 <sup>rd</sup> trifoliate leaf)
Second treatment application (phenophase) <sup>#</sup>	-	19.06.2020 (12 leaves)	09.06.2020 (8 leaves)	09.06.2020 (4 <sup>th</sup> trifoliate leaf)

\*Bio Enne contains: 12% organic nitrogen, 23% water soluble sulphuric anhydride, 35% organic carbon

<sup>#</sup>Foliar application; 2.5 L solution 0.5%/ha/treatment; applied volume 150 L.

### Biomass determinations

The biomass determinations were performed at maturity stage and are expressed as dry matter. For all cultures, the sampling was performed for 1 square meter as it follows: for wheat was used quadrat shape (1m x 1m), for sunflower

and maize were collected 5 plants (corresponding to 50000 plants/ha) and for soybean were collected plants from 2 rows of 1 m long and 0.50 m spacing between rows. After weighting whole plant, it was weighted

spikes, calatidium, cobs, siliques, seeds and the results were expressed as kg/ha of dry matter.

## RESULTS AND DISCUSSIONS

### 1. The efficiency of foliar fertilization with CODAMIX and ECOAMINOALGA on autumn wheat yield parameters

The experimental results (Table 5) indicated for wheat crop that all investigated yield parameters increased after foliar fertilization in comparison with control variant. It was noticed

a significant increase of total biomass and spikes biomass after F2 treatment, precisely with 47.45% and 47.71% respectively, as against control variant and with 28.77% and 34.40% respectively, as against F1 treatment. Also, all subjected parameters present superior values after F2 treatment than after F1 treatment and in both cases, higher than unfertilized (control) variant. Moreover, after F2 treatment, seeds biomass presented an increase with 53.78% as against control and with 20.89% in comparison with F1 treatment.

Table 5. The efficiency of foliar fertilization on autumn wheat yield parameters

Experimental variant (dose; number of treatments)	Total biomass, kg/ha	Spikes biomass, kg/ha	Seeds biomass, kg/ha	TKW, g
Control	4413	2293	1095	30.0
F1(2.5L/ha; 1)	5053	2520	1393	30.8
F2(2.5L/ha; 1)	6507*	3387*	1684	32.2
DL 5% =	1958	1013	678	5.1
DL 1 % =	3247	1681	1124	8.5
DL 0.1% =	6072	3144	2101	15.8

F1 = CODAMIX; F2 = ECOAMINOALGA; \*significant difference; \*\*distinct significant difference \*\*\*very significant difference.

### 2. The efficiency of foliar fertilization with CODAMIX and ECOAMINOALGA on sunflower yield parameters

The results concerning the sunflower crop, except those for TKW (Table 6) indicated significant and distinct significant differences between treatments and control variant. The values related to calatidium biomass, seeds biomass and TKW are higher after F1 treatment than after F2 treatment. Hence, after F1 treatment in comparison with control variant, these yield parameters increased with 9.38%,

10.80% and 12.00%, respectively. After F2, in comparison with control, the values for calatidium biomass and seeds biomass increased with 5.26% and 3.22%, respectively meanwhile TKW decreased with 4.65%. Concerning total biomass, the treatment F2 produced an increase with 23.75% and 8.92% as against control and F1, respectively. Nevertheless, application of F1 and F2 evidenced positive influence on all yield parameters.

Table 6. The efficiency of foliar fertilization on sunflower yield parameters

Experimental variant (dose; number of treatments)	Total biomass, kg/ha	Calatidium biomass, kg/ha	Seeds biomass, kg/ha	TKW, g
Control	10533	5700	3100	40.8
F1 (2.5L/ha; 2)	11967**	6235***	3435***	45.7
F2 (2.5L/ha; 2)	13035***	6000**	3200**	38.9
DL 5% =	475	104	57	12.5
DL 1 % =	787	172	94	20.7
DL 0.1% =	1473	322	177	38.7

F1 = CODAMIX; F2 = ECOAMINOALGA; \*significant difference; \*\*distinct significant difference \*\*\*very significant difference.

### 3. The efficiency of foliar fertilization with CODAMIX and ECOAMINOALGA on maize yield parameters

For maize crop, the results (excepting TKW parameter) indicated significant and distinct significant differences between treatments and

control variant (Table 7). Concerning the efficiency of applied inputs, the experimental results evidenced that in all cases, application of F2 generated upper values for all yield parameters than F1. The most important increase (71.38%) after F2 treatment in

comparison with control variant was recorded for total biomass parameter. After F2 treatment, cobs biomass increased with 29.50% and 22.87% in comparison with control and F1, respectively, meanwhile seeds biomass

presented similar increases: with 30% and 22.78% as against control and F1, respectively. TKW values after F2 treatment were with 10.81% and 3.14% higher in comparison with F1 and control variant, respectively.

Table 7. The efficiency of foliar fertilization on maize yield parameters

Experimental variant (dose; number of treatments)	Total biomass, kg/ha	Cobs biomass, kg/ha	Seeds biomass, kg/ha	TKW, g
<b>Control</b>	12370	7467	5667	318
<b>F1</b> (2.5L/ha; 2)	17669***	7870**	6000**	296
<b>F2</b> (2.5L/ha; 2)	21200***	9670***	7367***	328
DL 5% =	901	228	198	35
DL 1% =	1495	378	328	59
DL 0.1% =	2796	706	613	110

F1 = CODAMIX; F2 = ECOAMINOALGA; \*significant difference; \*\*distinct significant difference \*\*\*very significant difference.

#### 4. The efficiency of foliar fertilization with CODAMIX and ECOAMINOALGA on soybean yield parameters

Even if all yield parameters for soybean crop are higher after treatments with F1 and F2, respectively in comparison with control variant, the differences between them are very small. In

the case of siliques biomass and TKW parameters, it was found significant differences between treatments. For these two parameters in comparison with control were found increases as it follows: after F1 with 3.50% and 12.08%, respectively and after F2 with 4.28% and 15.38%, respectively.

Table 8. The efficiency of foliar fertilization on soybean yield parameters

Experimental variant (dose; number of treatments)	Total biomass, kg/ha	Siliques biomass, kg/ha	Seeds biomass, kg/ha	TKW, g
<b>Control</b>	4930	2570	1230	91
<b>F1</b> (2.5L/ha; 2)	5110	2660*	1280	102*
<b>F2</b> (2.5L/ha; 2)	5120	2680*	1290	105*
DL 5% =	104	85	76	9.5
DL 1% =	154	126	112	14.0
DL 0.1% =	238	195	173	21.6

F1 = CODAMIX; F2 = ECOAMINOALGA; \*significant difference; \*\*distinct significant difference \*\*\*very significant difference.

## CONCLUSIONS

The investigation of the efficiency of CODAMIX (F1) and ECOAMINOALGA (F2) (inputs accepted for organic agriculture) on different field crops (wheat, sunflower, maize, and soybean) evidenced positive effects on yield parameters in comparison with control variant.

For autumn wheat and maize, the values of all yield parameters evidenced the higher efficiency of F2 input in comparison with F1. Concerning sunflower crop, all yield parameters, excepting total biomass were higher after F1 treatment in comparison with F2. Also, excepting TKW after F2 treatment,

all parameters were higher after using both inputs in comparison with control variant.

For soybean, the effects of F1 and F2 inputs on yield parameters were similar, the results being close between F1 and F2 and slightly higher than those recorded for unfertilized variant.

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## EFFECTS OF DIFFERENT SOIL TILLAGE ON CASTOR BEAN CROP IN SOUTHERN ROMANIA

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### Abstract

*Castor bean (Ricinus communis L.) is a rustic, drought-resistant plant, with great adaptability to different climatic and soil conditions. Currently, it is known for the versatility of the oil extracted from its seeds, where expectations of rising demand for castor oil in the world market is high. In terms of the crop technology, it has to be considered that there are a number of variables for each growing area, especially when it is desired to adapt the castor bean crop at a certain soil tillage system. In this respect, the objective of the present paper is to put into evidence the effects of different soil tillage on the castor bean crop under the specific growing conditions from South Romania. In this respect, a field experiment was established in the years 2019 and 2020 at the Agricultural and Development Research Station Teleorman (ADRS Teleorman) located in South Romania (Teleorman county). The experimental variants were represented by the soil tillage with the following graduations: 1. Summer plowing performed at 30 cm depth + harrowing performed with a disc harrow in autumn, at 12 cm depth + 2 works of seedbed preparation performed with a seedbed cultivator in spring, before sowing, at 10 cm depth; 2. No tillage; 3. Harrowing performed with a disc harrow in spring, before sowing, at 15 cm depth; 4. Deep tillage performed with a ripper in autumn, at 35 cm depth + harrowing performed with a disc harrow for seedbed preparation in spring, before sowing, at 12 cm depth. The obtained results indicate that the conventional soil tillage is superior to the variants of minimum tillage, which implies giving up to plowing, or direct sowing, which implies no-tillage.*

**Key words:** castor bean, conventional tillage, minimum tillage, deep tillage, no-tillage.

### INTRODUCTION

Castor bean (*Ricinus communis* L.) is a rustic, drought-resistant plant, with great adaptability to different climatic and soil conditions. Currently, it is known for the versatility of the oil extracted from its seeds, where expectations of rising demand for castor oil in the world market is high. Despite the great importance of castor oil, it contributes to only 0.15% of the vegetable oil produced in the world (Severino et al., 2012; Patel et al., 2016).

Tillage is one of the important activities in the crop production system that optimizes the conditions of soil bed environment for seed germination, seedling establishment and crop growth (Wlaiwan and Jayasuriya, 2013). The tillage systems have evolved in recent decades, both in Romania and worldwide, and both conceptually and in terms of the extension of conservative tillage methods. The extension, in practice, of soil conservation tillage is different

from one country to another depending on the degree of mechanization and it increases with the increase of the tractors power and agricultural machineries capacity, as well as with the diversification of the equipment of loosening, tillage and sowing. Conservative soil tillage (minimum tillage and no-tillage) are considered among the most important components of conservation agriculture (Rusu et al., 2015). But, soil conservation systems in different areas must be differentiated, depending on the ecological characteristics of the area and the technological requirements of cultivated plants (Guş et al., 2004). Practically, choosing the good agricultural practices, especially related to the soil management, is a key factor in granting food, clean water, feed, energy, safe climate, diverse ecosystem services and biodiversity for future generations (Muşat et al., 2021).

The yields obtained, by applying the minimum tillage systems, show that differentiated results

can be obtained, the choice of the working variant in relation to the crop plant being decisive (Guş and Rusu, 2011). The results obtained in the countries where conservative agriculture has expanded show that it is of great importance for stopping soil degradation, leads to a good use of water from rainfall and irrigation, reducing climate effects, reducing costs and last but not least, increasing productivity (Sayre and Govaerls, 2010).

Traditional agriculture, based on intensive tillage by plowing with the return of furrow and removal of plant debris followed by numerous secondary works, has the disadvantage of high cost and disproportionate distribution of inputs from crop technology in relation to expected efficiency, low productivity as well as major risks regarding soil degradation and environmental pollution (Cociu, 2011). Actually, soil tillage is one of the greatest energy and labour consumer in a crop technology (Cociu, 2011; Ion et al., 2015). Many farmers are converting to reduce tillage systems to diminish soil erosion and field-work time requirements, and to remain eligible for government programs (Lund et al., 1993).

Regarding the technological level used in castor bean crop, as well as some cultural aspects, such as weed management, it is considered that there are a number of variables for each crop region, especially when it is desired to adapt castor bean crop to a certain system, as it is direct sowing (Maciel, 2006).

The new concepts regarding the way of cultivating the plants aiming at achievement of the yields at the level close to the biological potential of the cultivars, the conservation of the soil and the increase of the economic efficiency, are objectives pursued also in the case of castor bean cultivation.

Soils with loamy-sandy texture and soil acidity close to neutral are the soils on which castor bean plants grow best. Given the slow development of plants in the first part of the growing season and the pivoting root of castor bean, the soil should be well loosened in depth, avoiding soils where minimal work has been applied. After early preceding crops (as straw cereals), immediately after harvest, a work with a disc harrow or cultivator is performed and then a plowing is done at 22-30 cm depth. After maize or other crops harvested in late autumn,

plowing is carried out at a depth of 28-30 cm. The germination bed is prepared in spring, a few days before sowing, using a disc harrow or a seedbed cultivator, at the sowing depth (Sărdan, 2003).

Regarding the cultivation of castor bean in the direct sowing system, weed control is directly related to the obtained yield. In the conventional soil tillage systems, weeds are incorporated into the soil, these being destroyed and it being prevented the germination of their seeds or plant emergence from the depths. However, this practice must be performed following the technical criteria, otherwise it can lead to physical, chemical and biological degradation of the soil (Costa et al., 2013)

The adoption of soil and water conservation practices is an essential aspect in the rational exploitation of castor bean crop. This plant has a low leaf area index and is cultivated at greater distances than the main annual crops, leaving the soil between the rows unprotected, prone to erosive agents, which are wind (wind erosion) and rain (water erosion). In addition, castor bean plants export significant amounts from the soil to the detriment of the successive crops. Castor bean can deplete much of the soil nutrients in low-consumption production systems, further exacerbating the risk of erosion (Azevedo et al., 1997).

In terms of the crop technology, it has to be considered that there are a number of variables for each growing area, especially when it is desired to adapt the castor bean crop at a certain soil tillage system. In this respect, the objective of the present paper is to put into evidence the effects of different soil tillage on the castor bean crop under the specific growing conditions from South Romania.

## **MATERIALS AND METHODS**

Researches were carried out in field experiments at the Agricultural and Development Research Station Teleorman (ADRS Teleorman) located in South Romania (Teleorman county) in the years 2019 and 2020.

The researches were performed under rainfed conditions on a soil of cambic chernozem type, the vertical subtype. The soil has a loam-clay texture on the depth of the ploughed layer (0-

25 cm), this being characterized by a clay content of 45%, humus content of 3.1%, weakly acid soil reaction (pH varies between 6.1 and 6.5), total nitrogen content of 0.166%, mobile phosphorus content of 40-60 ppm, and mobile potassium content of 250 ppm. The main hydro-physical indices of the soil on the horizon 0-80 cm have the following average values: bulk density of 1.43 t/m<sup>3</sup>, field capacity of 27.3% (310.4 mm), and permanent wilting point of 15.0% (171.0 mm).

**Experimental design.** The experimental variants were represented by the soil tillage with the following graduations:

1. Summer *plowing* performed at 30 cm depth + harrowing performed with a disc harrow in autumn, at 12 cm depth + 2 works of seedbed preparation performed with a seedbed cultivator in spring, before sowing, at 10 cm depth;
2. *No tillage*;
3. *Harrowing* performed with a disc harrow in spring, before sowing, at 15 cm depth;
4. *Deep tillage* performed with a ripper in autumn, at 35 cm depth + harrowing performed with a disc harrow for seedbed preparation in spring, before sowing, at 12 cm depth.

Given the fact that sowing is difficult to be performed manually on different tillage systems, the experiment was performed on large plots, respecting all the rules of rigor applied to small plots.

The surface of the plot was 300 m<sup>2</sup> (L = 50 m, l = 6 m).

**Crop management.** The preceding crop was common autumn wheat.

In the autumn, 100 kg of nitrocalcar (27% nitrogen) were applied for all the experimental variants (for the experimental variant with plowing the application was made before harrowing performed in autumn and for the variant with deep tillage the application was made before this work). In the spring, before seedbed preparation for the experimental variants with plowing, harrowing and deep tillage, and before sowing for the experimental variant with no tillage, a complex chemical fertilizer of 15:15:15 type was applied, in a dose of 200 kg commercial product on ha.

All studied variants were sown on 26 of April in 2019, respectively on 20 of April in 2020.

The sowing was performed mechanized, for direct sowing with the Fabimag FG-01 universal seed drill, and for the other variants with the Romanian seed drill SPC-9. The sowing density was of 60,000 germinating seed on ha, the row spacing was of 70 cm, and the sown variety was Rivlas (mid-late variety created in Romania at ADRS Teleorman).

The control of the weeds was performed by the application immediately after sowing of the herbicide Dual Gold 960 EC (S-metolachlor 960 g/l) at a rate of 1.5 l/ha and Roundup Classic Pro (glyphosate 360 g/l) at a rate of 2.0 l/ha. For controlling of the monocotyledonous weeds in the vegetation period, the herbicide Leopard 5 EC (quizalofop-P-ethyl 50 g/l) was applied in a rate of 0.75 l/ha in the growth stage of 5-6 leaves. In our field experiments the control of dicotyledonous weeds in the vegetation period was done by a mechanical hoeing followed by a manual correction hoeing, except for the experimental variant with no tillage for which no mechanical hoeing was performed.

During the vegetation period, no phytosanitary treatments were performed, being necessary to note the reaction of castor bean plants to the appearance of the specific diseases and pests.

Harvesting was done manually. After harvesting, the seeds were peeled by hand on each variant. The seed yield was calculated on hectare and it was reported at 9% moisture content.

The percentage of oil in the seeds was determined based on the magnetic resonance phenomenon performed on the Spinlock device.

The productivity elements were evaluated at 10 plants chosen at random from each experimental variants.

The calculation and interpretation of the results was done based on the analysis of variance (Săulescu and Săulescu, 1967).

**Climatic data.** In terms of temperature in the experimental years, castor bean plants benefited throughout the vegetation period from temperatures higher than the multiannual average value (Figure 1).

In terms of water, in 2019, castor bean plants benefited from 376.6 mm of rainfall over the entire vegetation period, this being with 76.6 mm more than the crop's requirements for

moisture, but their distribution was unfavorable to the castor bean crop. Thus, in the first part of the vegetation period the precipitations were quantitatively higher than the multiannual average value by 27.2 mm in April, by 48.1 mm in May, and by 99.3 mm in June. During the period of the plant yield components formation, respectively in July and August, there was an accentuated water deficit of 27.1 mm in July and of 47.2 mm in August, a month in which no rainfall was registered (Figure 2).

In 2020, there were excess rainfall in May (+7.8 mm) and June (+11.6 mm), while in April, July and August a cumulative deficit of 92.9 mm was registered, compared to the multiannual averages of the area. In July, it can be said that the drought was installed, when only 2.8 mm of rainfall was recorded, the rainfall being practically absent. In August, 12.6 mm of rainfall was recorded, of which 12.2 mm in the second decade.

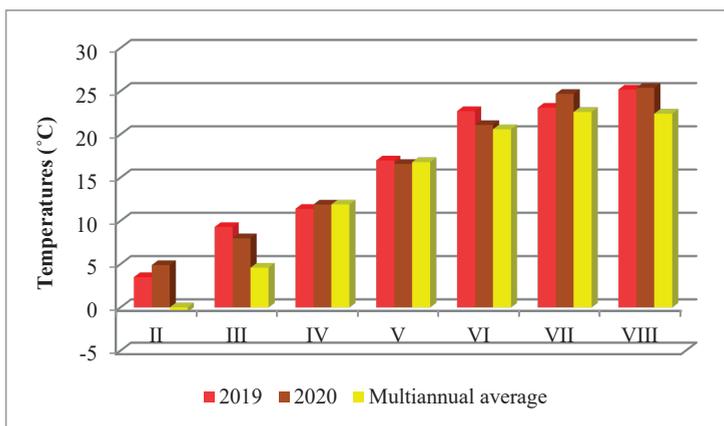


Figure 1. Evolution of the average monthly temperatures at ARDS Teleorman in the years 2019 and 2020

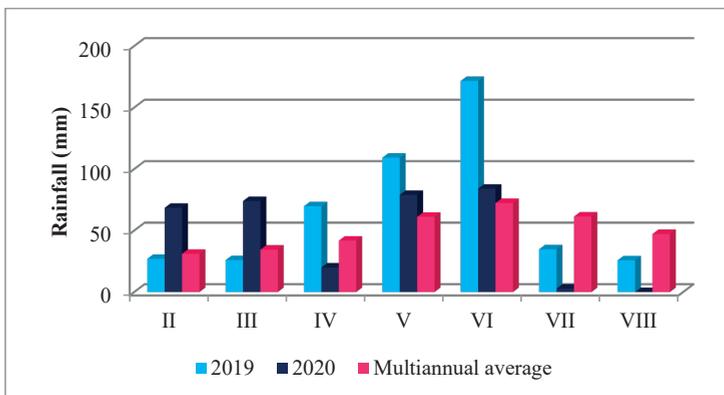


Figure 2. Evolution of the rainfall at ARDS Teleorman in the years 2019 and 2020

## RESULTS AND DISCUSSIONS

The castor bean plants emerged in 12 days for the experimental variants with plowing and deep tillage, both in 2019 and 2020 (Table 1). The soil tillage performed only by harrowing delayed the plant emergency by 2 days in 2019 (when the plants emerged in 14 days) and by

4 days in 2020 (when the plants emerged in 16 days). But, in the case of the variant with no-tillage, the emergence period was almost double compared to variants with plowing and deep tillage, the castor bean plants emerging in 22 days in 2019 and in 24 days in 2020.

Following the phenological observations made in 2019, no plants attacked by *Fusarium ricini*,

*Botrytis cinerea* and *Macrosporium ricini* were identified (Table 2), but an attack of *Xantomonas ricinicola* was identified in all experimental variants in a percentage of 4-10% of plants attacked per variant. In 2020, due to unfavorable climatic conditions for the development of pathogens, the presence of any of the pathogens mentioned above was not identified.

The percentage of emerged plants by variants had significant differences as follows (Table 3). The variant with plowing registered the highest emergence percentage reported to the sown germinating seeds, respectively 92.7% in 2019 and 90.6% in 2020. The variant with deep tillage was close to the variant with plowing in 2019, registering an emergence percentage of 91.4%, but in 2020 it registered only 76.9%. The variant with no-tillage registered the smallest emergence percentage, respectively

61.1% in 2019 and 71.6% in 2020, while the variant with harrowing was close to the variant with no-tillage, registering an emergence percentage of 71.6% in 2019 and of 75.6% in 2020.

For the control of dicotyledonous weeds there is not yet an herbicide for the castor bean crop, reason for which the weed control was done by a mechanical hoeing followed by a manual correction hoeing for all experimental variants, except for the experimental variant with no tillage for which no mechanical hoeing was performed. In the case of variant with harrowing, after the mechanical plowing, the phenomenon of uprooting the plants was observed. This phenomenon was observed also in the case of the variant with no-tillage, this occurring due to the fact that the root could not grow in the depth of the soil, instead it developed in the surface layer of the soil.

Table 1. Emergence of the castor bean plants at different soil tillage (ARDS Teleorman, 2019 and 2020)

Experimental variant	2019		2020		Average no of days for emergence
	Date	No of days for emergence	Date	No of days for emergence	
1. Plowing	8 of May	12	2 of May	12	12
2. No tillage	18 of May	22	14 of May	24	23
3. Harrowing	10 of May	14	6 of May	16	15
4. Deep tillage	8 of May	12	2 of May	12	12
Average	-	15	-	16	15.5

Table 2. Disease resistance (notes: 1 - resistant ... 9 - sensible) of the castor bean plants at different soil tillage (ARDS Teleorman, 2019 and 2020)

Experimental variant	<i>Fusarium ricini</i>		<i>Botrytis cinerea</i>		<i>Macrosporium ricini</i>		<i>Xantomonas ricinicola</i>	
	2019	2020	2019	2020	2019	2020	2019	2020
1. Plowing	1	1	1	1	1	1	2	1
2. No tillage	1	1	1	1	1	1	3	1
3. Harrowing	1	1	1	1	1	1	2	1
4. Deep tillage	1	1	1	1	1	1	2	1

Table 3. Plant density and percentage of broken plants at different soil tillage (ARDS Teleorman, 2019 and 2020)

Experimental variant	Sowing (germinating seeds/ha)	Plant density								Broken plants (%)	
		Emergence				Harvesting				2019	2020
		2019		2020		2019		2020			
		2019/2020	plants/ha	%*	plants/ha	%*	plants/ha	%**	plants/ha	%**	
1. Plowing	60,000	55,637	92.7	54,334	90.6	47667	85.7	48667	89.6	13.2	1.7
2. No tillage	60,000	36,667	61.1	42,952	71.6	25000	68.2	25903	60.3	31.8	4.3
3. Harrowing	60,000	42,978	71.6	45,375	75.6	39333	91.5	30750	67.8	19.4	2.3
4. Deep tillage	60,000	54,823	91.4	46,134	76.9	43578	79.5	32267	69.9	16.1	2.0
Average	60,000	47,526	79.2	47,199	78.7	38,895	81.2	34,397	71.9	20.1	2.6

\*Emergence percentage is calculated reported to the germinating seeds.

\*\*The percentage of plants at harvest is calculated reported to the number of plants at emergence.

After the phenological phase of the appearance of the main raceme, it was no longer possible to enter in the crop with agricultural equipment to control weeds, due to the size of the plants.

In the variants with plowing and deep tillage, due to the higher plant density but also due to the castor bean plant vigor, the weeds could no longer develop and they were no longer a problem for castor bean plants. In contrast, in the case of variant with harrowing but especially in the case of variant with no-tillage, weeds were a fierce competitor for nutrition space. Thus, castor bean plants were poorly developed and grown. An impediment in the development of castor bean plants, in 2019, was also represented by the amount of precipitation that fell during the period of vegetative growth, when the weeds grew at the same time as the castor bean plants, even exceeding them.

Regarding the percentage of broken plants, the same tendency can be observed as in the case of plant density (Table 3). Thus, for the variant with plowing the percentage of broken plants was the lowest (13.2% in 2019 and 1.7% in 2020), while in the case of variant with no-tillage the percentage of broken plants was more than double (31.8% in 2019 and 4.3% in 2020). This phenomenon is explained by the fact that castor bean plants have had a deficient

development in the variants with minimal soil works.

Biometric determinations of morphological elements show the same differences as in the case of plant density and percentage of broken plants. Thus, it can be observed that all biometric characters (number of nodes/plant, number of branches/plant, plant height, insertion height of the main raceme, and length of the main raceme) have the highest values in the case of variant with plowing and the lowest values in the case of variant with no-tillage (Tables 4 and 5). The variant with deep tillage is close to the variant with plowing, while the variant with harrowing is close to the variant with no-tillage. Following these results, it can be concluded that castor bean plants find the best conditions for growth and development when sown is performed in a soil worked by summer plowing + harrowing with a disc harrow in autumn + seedbed preparation performed with a seedbed cultivator in spring, and a comparable situation can be registered in the case of deep tillage performed with a ripper in autumn + harrowing performed with a disc harrow for seedbed preparation in spring. The direct sown, respectively the conditions of no-tillage assure less favorable growing conditions for castor bean plants, a comparable situation being registered for the case of the soil tillage performed only by harrowing.

Table 4. Number of nodes and branches on castopr bean plant and plant height at different soil tillage (ARDS Teleorman, 2019 and 2020)

Experimental variant	Number of nodes/plant			Number of branches/plant			Plant height (cm)		
	2019	2020	Average	2019	2020	Average	2019	2020	Average
1. Plowing	9	8	8.5	2	1	1.5	130	117	123.5
2. No tillage	7	6	6.5	1	1	1.0	109	105	107.0
3. Harrowing	8	8	8.0	1	1	1.0	119	108	113.5
4. Deep tillage	8	8	8.0	2	1	1.5	130	117	123.5
Average	8	7.5	7.75	1.5	1	1.25	122	111.8	116.9

Table 5. Insertion height of the main raceme and its length at different soil tillage (ARDS Teleorman, 2019 and 2020)

Experimental variant	Insertion height of the main raceme (cm)			Length of the main raceme (cm)		
	2019	2020	Average	2019	2020	Average
1. Plowing	80	90	85.0	50	30	40.0
2. No tillage	67	80	73.5	38	19	28.5
3. Harrowing	75	81	78.0	40	27	35.5
4. Deep tillage	80	90	80.5	48	29	38.5
Average	75.5	85.25	79.25	44	26.25	35.63

As in the case of morphological elements of the castor bean plants, the productivity elements of the plants (number of capsules on main raceme, weight of capsules on main raceme, number of seeds on main raceme, weight of seeds on main raceme, and TGW - Thousand Grain weight) varied depending on the soil tillage, the variant with plowing recording the highest values, compared to the other studied soil tillage

variants (Tables 6 and 7). Also, the smallest values of the productivity elements were registered in the case of the variant with no-tillage.

The oil content of the seeds varied, on average over the years of experimentation, from 53.5% in the case of variant with plowing to 47.1% in the case of variant with no-tillage (Table 7).

Table 6. Number of capsule, weight of capsules and number of seeds on main raceme at different soil tillage (ARDS Teleorman, 2019 and 2020)

Experimental variant	Number of capsule on main raceme			The weight of capsules on main raceme (g)			Number of seeds on main raceme		
	2019	2020	Average	2019	2020	Average	2019	2020	Average
1. Plowing	72	51	61.5	80.5	58.6	69.6	165	93	129.0
2. No tillage	67	46	56.5	53.2	51.4	53.3	135	85	110.0
3. Harrowing	64	50	57.0	60.4	55.6	58.0	130	91	110.5
4. Deep tillage	62	51	56.5	62.8	57.9	60.4	135	93	114.0
Average	66.3	49.5	57.9	64.2	55.9	60.3	141.3	90.5	115.9

Table 7. The weight of seeds on main raceme, TGW and oil content of the seeds at different soil tillage (ARDS Teleorman, 2019 and 2020)

Experimental variant	The weight of seeds on main raceme (g)			TGW (g)			Oil content of the seeds (%)		
	2019	2020	Average	2019	2020	Average	2019	2020	Average
1. Plowing	53.3	29.3	41.3	323	318	320.5	53.8	53.2	53.5
2. No tillage	39.7	21.8	30.8	294	256	275.0	46.9	47.2	47.1
3. Harrowing	37.9	28.9	33.4	292	271	281.5	49.6	50.7	50.2
4. Deep tillage	39.9	29.3	36.3	296	300	298.0	50.9	51.3	51.1
Average	42.7	27.3	35.5	301.3	286.3	293.8	50.3	50.6	50.5

The seed yields obtained, on average over the years of experimentation, were of 1937 kg/ha for the variant with plowing, 1335 kg/ha for the variant with deep tillage, 921.5 kg/ha for the variant with harrowing, and 750 kg/ha for the variant with no-tillage (Table 8). So, the highest seed yields were registered in the case of variant with plowing, while the smallest seed yields were registered in the case of the variant with no-tillage. The large differences in yields are explained by the differences in plant density

at harvest, but also by the way of growth and development of plants throughout the vegetation period according to the soil tillage variant.

The less favorable growing conditions of the year 2020, especially related to the drought registered in this year, affected considerable the yielding capacity of the castor bean plants, the average seed yield of this year being of 888.3 kg/ha, compared to the seed yield registered in the year 2019 of 1583.5 kg/ha.

Table 8. Seed yields obtained at different soil tillage (ARDS Teleorman, 2019 and 2020)

Experimental variant	Seed yields (kg/ha)			Relativ seed yields (%)	Difference (kg/ha)	Significance
	2019	2020	Average			
1. Plowing	2454	1420	1937	100	Control	-
2. No tillage	980	520	750	38.7	-1187.0	<sup>000</sup>
3. Harrowing	1120	723	921.5	47.6	-1015.5	<sup>000</sup>
4. Deep tillage	1780	890	1335	68.9	-602.0	<sup>000</sup>
Average	1583.5	888.3	1235.9	-	-	-

LSD5% = 150.48 kg/ha; LSD1% = 227.87 kg/ha; LSD0.1% = 366.07 kg/ha

The technological elements can influence the way of capitalization of the water from precipitation.

In order to highlight the role of precipitation in crop formation depending on soil tillage, the precipitation recovery coefficient was calculated (kg of produced seeds/mm precipitation), relating the yields obtained to

the amount of precipitation during the growing period of the castor bean plants.

Analyzing the data from the Table 9, we can see that the rainwater is best used when the sowing is performed in a soil worked by summer plowing + harrowing with a disc harrow in autumn + seedbed preparation performed with a seedbed cultivator in spring.

Table 9. Coefficient of recovery of water from precipitation depending on soil tillage (ARDS Teleorman, 2019 and 2020)

Experimental variant	Coefficient of precipitation recovery (kg of produced seeds/mm)		%		Difference (kg/ha)		Significance	
	2019	2020	2019	2020	2019	2020	2019	2020
1. Plowing	5.97	7.64	Control		100		-	
2. No tillage	2.38	2.80	39.93	36.62	-3.58	-4.84	0	00
3. Harrowing	2.72	3.89	45.64	50.92	-3.24	-3.75	0	0
4. Deep tillage	4.33	4.79	72.53	62.68	-1.64	-2.85		0

For 2019: LSD5% = 2.80 kg/mm; LSD1% = 4.64 kg/mm; LSD0.1% = 8.68 kg/mm  
 For 2020: LSD5% = 2.60 kg/mm; LSD 1% = 4.30 kg/mm; LSD0.1% = 8.05 kg/mm

## CONCLUSIONS

Castor bean is an exigent plant in land preparation. The obtained results indicate that the conventional soil tillage (involving plowing, a harrowing in autumn performed with a disc harrow and the seed bed preparation in spring performed with a seedbed cultivator) is superior to the variants of minimum tillage, which implies giving up to plowing, or direct sowing, which implies no-tillage.

In the case of minimum tillage without deep tillage (based on harrowing performed in spring), but especially in the case of no-tillage, the seed yield of the castor bean crop can be smaller by over 1000 kg/ha compared to the conventional soil tillage.

In the case of giving up at plowing and having a deep tillage in autumn and a harrowing in spring for seedbed preparation, the seed yield at castor bean is between those obtained in the case of conventional soil tillage and those obtained in the case of minimum tillage based on harrowing performed in spring. The variant with a deep tillage in autumn and a harrowing in spring for seedbed preparation could be of interest especially in situations when the plowing cannot be performed in summer or in autumn, as for example in drought conditions.

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## FINITE ELEMENT AND APPLIED MODELS OF THE STEM WITH SPIKE DEFORMATION

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### Abstract

*Considered by this article are static and dynamic deformation of the stem with the ear of wheat and of the individual grain. The purpose of the article is to determine the factors of influence on the ear of wheat to isolate the grain. The ear fluctuations are considered within the Euler-Bernoulli bar theory. The first stage studies the dynamics of the plant as a whole, the second stage studies the dynamics of an individual grain in a moving system associated with the ear. The model of the ear and the grain fluctuation uses the mechanical characteristics of elastic bonds and elastic bodies, which are determined by spring stiffness's, elastic moduli, etc. The natural frequencies shows that at the value of natural frequencies of grain fluctuations 131,6 Hz and 1274,9 Hz the grain itself fluctuates predominantly, and at frequencies 1622,8 Hz, 7367.3 Hz, 9396.0 Hz and 13491,0 Hz the flakes vibrate.*

**Key words:** early stages of maturation, ear fluctuations, grain of wheat, harvest, mathematical model.

### INTRODUCTION

Agriculture was and is the most important sector of our country's national economy. Agribusiness must meet the growing needs, on the one hand, of the population for food, and, on the other hand, of the industry - for raw materials (Andruszczak P.K.S. et al., 2019; Berihuete-Azorin M. et al., 2020). Modern production implies a highly efficient use of the production potential of the agro-industrial complex; technical, quality and, especially, reliability upgrade of agricultural machinery; reducing losses and improving the quality of agricultural products.

Modern agriculture is unthinkable without the cultivation and production of grain crops. Grain crops occupy an area over 40 million hectares in the Russian Federation, which is 10% of the world's land area. Grain is the main and indispensable product in the human diet as well as productive farm animals and the main element of food security (Pakhomov V. et al., 2015; 2016; Rybas I. et al., 2016). Grain crops have high nutritional, taste and dietary properties. Grain production achievements, are essentially one of the most important indicators

of the development level of agriculture in general.

The leading role among grain crops belongs to wheat and barley. They occupy more than half of the global acreage. This explains the close attention to all issues in one way or another related to the production and harvesting of these crops (Buryanov M. et al., 2015a; Rykov V. et al., 2016; Sokolova E. et al., 2020).

Plants of these crops at the maturation time contain a grain in the ear, which, in natural conditions, in the full ripeness phase gradually loses mechanical connection with it and crumbles in to the field. For the harvest of full-grown grain, it is necessary to carry out timely harvesting before it falls off and artificially force grain separation from the ear (Bello R.S., 2012; Buryanov A. et al., 2015b).

Combine harvesters are now used everywhere for harvesting. Up to now, they are the main machine for crops' harvesting. Their designs implement a method of threshing and primary separation of the harvested heap. This method, invented more than a century ago, involves cutting the entire crop and feeding it into a threshing machine, where it is subjected to the drum beaters impact while being dragged

through a rigid grid. Up to 80% of the power is spent on threshing and grinding the straw, and about 7% of the energy is used to separate the grain from the receptacle.

This impact mode on the processed mass in combines, necessary for the extraction of grain and its separation from the ears of plants through the deck, has serious draw-backs. First, it is energy-consuming. Moreover, secondly, it leads to an overall high level of grain injury up to 20-30% of the entire threshed mass of grain.

Injured grain has reduced germination, and pathogens develop in the resulting cracks during storage. This often makes it impossible to obtain quality seed material, and when a certain level of concentration of microtoxins is reached, the grain is unsuitable not only for the production of baked goods, but also for animal feed.

Moreover, the self-drainage process can occur in grain crops due to insufficient connection of the grain to the ear during untimely harvesting (Buryanov A. et al., 2014). This also causes grain losses, which amount to millions of tons per year.

Thus, pressing issue of the day is to ensure low-energy and low-traumatic forced grain extraction from the ear.

For this purpose, it is necessary not only to improve the existing methods and ways of grain harvesting (Kolesnikov D.A. et al., 2010; Miu P., 2015) but also to develop new highly effective physical-mechanical methods of its harvesting and, accordingly, to design the appropriate equipment (Lachuga U. et al., 2014; Lysenok O. & Matrosov A, 2014; Korotky A. et al., 2019; Ivanov V. et al., 2020). The solution of this problem, on the one hand, requires the development and creation of adequate analytical and numerical physical-mechanical models, including models based on CAD-CAE complexes, which are able to describe the interaction between the elements of the stem-column-grain system. At the same time, on the other hand, there are physical-mechanical models of the process of working bodies of grain harvesting equipment impact on the system stem - spike - grain (Lachuga F. et al., 2019a; Meskhi B. et al., 2019; Pakhomov V. et al., 2020).

## MATERIALS AND METHODS

A block theory can be considered as an approximate mathematical model considering the peculiarities of the geometry. Herewith, in general case, the section characteristics and mechanical properties depend on the longitudinal coordinate of the beam, which leads to boundary value problems with variable coefficients that can be solved analytically only in some particular cases. A simpler model is a beam consisting of two homogeneous parts (stem and spike) in this case the solution could be constructed analytically in both static and harmonic analysis.

### Ear and grain fluctuation mathematical model based on the beam theory

**Continuous one-dimensional ear fluctuation model.** Ear fluctuations are considered within the Euler-Bernoulli beam theory. The ear model is represented by an inhomogeneous beam with variable cross-section, density, and elastic modulus (Figure 1).

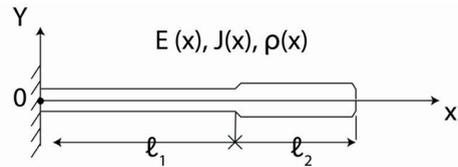


Figure 1. Ear diagram

The lower end of the rod is fixed, a distributed pressure or a concentrated force act on its surface, which in general case depend on time variable, and in special case it is a harmonic influence with circular frequency. The fluctuation equation has the form with respective transverse displacements and appears as following:

$$\frac{\partial^2}{\partial x^2} \left( E(x)J(x) \frac{\partial^2 v}{\partial x^2} \right) + \rho(x) \frac{\partial^2 v}{\partial t^2} = q(x, t) \quad (1)$$

Where:  $E(x)$  - Young's modulus,  $J(x)$  - moment of inertia,  $\rho(x)$  - density.

Boundary conditions:

$$\text{at } x=0 \quad v=0, \quad \frac{\partial v}{\partial x}=0$$

$$\text{at } x = l_1 + l_2, \quad \frac{\partial^2 v}{\partial x^2} = 0, \quad \frac{\partial^3 v}{\partial x^3} = 0 \quad (2)$$

Initial conditions correspond to the absence of motion at the initial moment of time:

$$v(x,0) = 0, \quad \frac{\partial v}{\partial t} \Big|_{t=0} = 0 \quad (3)$$

In general case parameters' dependence of the ear model on the axial coordinate, the solution of the initial boundary value problem 1-3 can be constructed numerically, particular in computer mathematical systems such as Maple or MatLAB mathematical packages, using the built-in numerical integration procedures of differential equations.

According to Figure 1, the rod consists of two main parts, where, in a simplified version, its characteristics can be considered constant. In this case, the mathematical model (4) consists of a differential equations system with constant coefficients and boundary conditions, presented in formula (2), has the following form:

$$E_i J_i \frac{\partial^4 v_i}{\partial x^4} + \rho_i \frac{\partial^2 v_i}{\partial t^2} = q(x,t) \quad i = 1,2 \quad (4)$$

Where:  $E_i$  - Young's modulus,  $J_i$  - moment of inertia,  $\rho_i$  - density, which are constant values at each site.

And the docking equations at:

$$v_1 = v_2, \quad \frac{\partial v_1}{\partial x} = \frac{\partial v_2}{\partial x}$$

$$E_1 J_1 \frac{\partial^2 v_1}{\partial x^2} = E_2 J_2 \frac{\partial^2 v_2}{\partial x^2}, \quad E_1 J_1 \frac{\partial^3 v_1}{\partial x^3} = E_2 J_2 \frac{\partial^3 v_2}{\partial x^3} \quad (5)$$

For harmonic excitation of fluctuations with a circular frequency, the system of equations (4) respecting the amplitude of fluctuations will look like this:

$$E_i J_i \frac{\partial^4 v_i}{\partial x^4} + \rho_i \omega^2 v_i = q(x) \quad (6)$$

The homogeneous ( $q(x) \equiv 0$ ) boundary value equation (6), (2), (5) are considered to find the resonant natural frequencies.

**Relative grain motion equations.** Figure 2 shows the layout of the grain in question and the mobile coordinate system  $O_1 x_1 x_2 x_3$ .

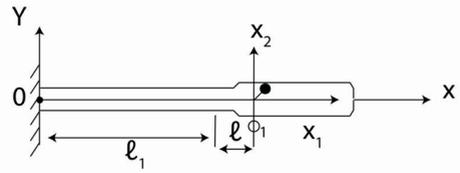


Figure 2. Local (mobile) coordinate system

Figure 3 shows the scheme of grain attachment to the ear, 3 a) - on the stem, 3 b) - without stem. Grain dynamics is investigated in two variants: the first model does not take into account grain size and considers a material point; the second model considers a solid body with a given inertia matrix.

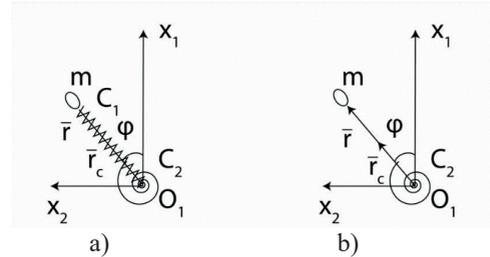


Figure 3. Grain fixing scheme

The linear stiffness spring  $C_1$  and the spiral stiffness spring  $C_2$  simulate grain and ear relationship, moreover the linear spring corresponds to the stem presence and the spiral spring corresponds to the scales interaction.

Choosing a mobile coordinate system coherent with the translational transverse motion of the point ( $x = l_1 + l$ ) of grain to ear attachment, grain acceleration can be represented as follows:

$$\bar{a} = \bar{a}_e + \bar{a}_r \quad (7)$$

Where:  $\bar{a}_e$ ,  $\bar{a}_r$  - transfer acceleration and the relative acceleration, respectively:

$$\bar{a}_e = \left( 0, \frac{\partial^2 v}{\partial t^2} \Big|_{x=l_1+l}, 0 \right) \quad (8)$$

Herewith transport acceleration is found from the solution of the corresponding boundary value problems of Section 2.1.

Then the motion of the grain equation with mass  $m$  in the mobile coordinate system  $O_1 x_1 x_2 x_3$  with initial conditions will take the form:

$$m\ddot{\vec{r}} = \vec{F} - m\vec{a}_e \quad (9)$$

$$\vec{r}|_{t=0} = \vec{r}_c, \quad \dot{\vec{r}}|_{t=0} = 0$$

Where:  $\vec{r} = (x_1, x_2, x_3)$  - radius vector of the grain mass center in motion,  $\vec{r}_c$  - radius vector of the grain mass center before motion commencement,  $\vec{F} = \vec{F}_p + \vec{F}_1 + \vec{F}_2$  the resultant force of the weight and elasticity forces of the linear and circular springs respectively:

$$\vec{F}_p = -(mg, 0, 0), \quad \vec{F}_1 = -C_1 \|\vec{r}\| - |\vec{r}_c| \|\vec{r}\| / |\vec{r}|,$$

$$\vec{F}_2 = \frac{C_2(\varphi - \varphi_0)}{|\vec{r}|} (\sin \varphi, -\cos \varphi, 0), \quad \varphi = \arctan(x_2/x_1) \quad (10)$$

When moving in the plane  $O_1x_1x_2$ , the vector equation (9) is reduced to a system (taking into account the notations  $x_1 = x(t)$ ,  $x_2 = y(t)$ ).

$$\begin{aligned} \frac{d^2}{dt^2}x(t) &= -\frac{C_1| - 1 + \frac{\sqrt{x_0^2 + y_0^2}}{\sqrt{x(t)^2 + y(t)^2}}| x(t)}{m} + \frac{C_2 \left( \arctan\left(\frac{y(t)}{x(t)}\right) - \arctan\left(\frac{y_0}{x_0}\right) \right) y(t)}{\sqrt{x(t)^2 + y(t)^2} x(t) \sqrt{1 + \frac{y(t)^2}{x(t)^2}} m} \\ \frac{d^2}{dt^2}y(t) &= -\frac{C_1| - 1 + \frac{\sqrt{x_0^2 + y_0^2}}{\sqrt{x(t)^2 + y(t)^2}}| y(t)}{m} + \frac{C_2 \left( \arctan\left(\frac{y(t)}{x(t)}\right) - \arctan\left(\frac{y_0}{x_0}\right) \right)}{\sqrt{x(t)^2 + y(t)^2} \sqrt{1 + \frac{y(t)^2}{x(t)^2}} m} + \omega^2 V_0 \sin(\omega t) \end{aligned} \quad (11)$$

Where:  $x_0, y_0$  - initial coordinates of the grain mass center,  $\omega$  - fluctuations' circular frequency of the ear,  $V_0$  - point fluctuations' amplitude  $O_1$  center of grain attachment (Figure 3).

Equations 9 and 10 are nonlinear differential equations and its solutions can be numerically constructed. As a result, maximum elastic force  $F_1$ , moment of elasticity  $M_2 = C_2\varphi$ , and their effect  $A_1, A_2$  in the increasing section  $|\vec{r}|$  and  $|\varphi|$ , can be found

$$A_1 = -C_1 \frac{\|\vec{r}_{\max}\| - |\vec{r}_c|}{2}, \quad A_2 = -C_2 \frac{\varphi_{\max}^2}{2} \quad (12)$$

$$\begin{aligned} y_1(x) &= C_1 \left( \frac{1}{2} \cosh(\beta_1 x) + \frac{1}{2} \cos(\beta_1 x) \right) + C_2 \left( \frac{1}{2} \sin(\beta_1 x) + \frac{1}{2} \sinh(\beta_1 x) \right) \\ &+ C_3 \left( \frac{1}{2} \cosh(\beta_1 x) - \frac{1}{2} \cos(\beta_1 x) \right) + C_4 \left( \frac{1}{2} \sin(\beta_1 x) + \frac{1}{2} \sinh(\beta_1 x) \right) \end{aligned} \quad (13)$$

$$\begin{aligned} y_2(x) &= C_5 \left( \frac{1}{2} \cosh(k\beta_1 x) + \frac{1}{2} \cos(k\beta_1 x) \right) + C_6 \left( \frac{1}{2} \sin(k\beta_1 x) + \frac{1}{2} \sinh(k\beta_1 x) \right) \\ &+ C_7 \left( \frac{1}{2} \cosh(k\beta_1 x) - \frac{1}{2} \cos(k\beta_1 x) \right) \\ &+ C_8 \left( \frac{1}{2} \sin(k\beta_1 x) + \frac{1}{2} \sinh(k\beta_1 x) \right) \end{aligned} \quad (14)$$

In turn these are compared to similar experimental data related to grain extraction from the ear, obtained in experiments on grain extraction from the ear (Shack Yu. et al., 2013; Lachuga Y. et al., 2019b; Matrosov M. et al., 2020).

**Analytical solution to the problem.** The solution of the homogeneous boundary value problem (6), (2), (5) will be found in the form (here and below  $v_1(x), v_2(x)$  denoted by  $y_1(x), y_2(x)$ , a  $l_1, l_2$  and by  $L_1, L_2$  respectively)

where, to simplify the notation, we use the following  $\beta_1 := \frac{p_1 \omega^2}{(E_1 J_1)^{3/4}} L_1$ ,  $k := \frac{p_2 E_1 J_1}{p_1 E_2 J_2} \frac{L_1}{L_2}$   $\cosh(\cdot)$  and  $\sinh(\cdot)$  - are hyperbolic cosine and sine, respectively. The constants  $C_1, C_2, \dots, C_8$  are found further from the boundary and coupling conditions.

Satisfying the boundary conditions at the ends of the beam we obtain

$$C_1 := 0, C_2 := 0, C_7 := 0, C_8 := 0 \quad (15)$$

The docking conditions (5) lead to a linear system:

$$\begin{aligned} & C_3 \left( \frac{1}{2} \cosh(\beta_1 L_1) - \frac{1}{2} \cos(\beta_1 L_1) \right) + C_4 \left( -\frac{1}{2} \sin(\beta_1 L_1) + \frac{1}{2} \sinh(\beta_1 L_1) \right) \\ & - C_5 \left( \frac{1}{2} \cosh(k\beta_1 L_2) + \frac{1}{2} \cos(k\beta_1 L_2) \right) - C_6 \left( \frac{1}{2} \sin(k\beta_1 L_2) + \frac{1}{2} \sinh(k\beta_1 L_2) \right) = 0 \\ & C_3 \left( \frac{1}{2} \sinh(\beta_1 L_1) \beta_1 + \frac{1}{2} \sin(\beta_1 L_1) \right) \beta_1 + C_4 \left( -\frac{1}{2} \cos(\beta_1 L_1) \beta_1 + \frac{1}{2} \cos h(\beta_1 L_1) \right) \beta_1 \\ & + C_5 \left( \frac{1}{2} \sinh(k\beta_1 L_2) k \beta_1 - \frac{1}{2} \sin(k\beta_1 L_2) k \beta_1 \right) \\ & + C_6 \left( \frac{1}{2} \cos(k\beta_1 L_2) k \beta_1 + \frac{1}{2} \cosh(k\beta_1 L_2) \right) k \beta_1 = 0 \\ & C_3 \left( \frac{1}{2} \cosh(\beta_1 L_1) \beta_1^2 + \frac{1}{2} \cos(\beta_1 L_1) \right) \beta_1^2 + C_4 \left( \frac{1}{2} \sin(\beta_1 L_1) \beta_1^2 + \frac{1}{2} \sin h(\beta_1 L_1) \right) \beta_1^2 \\ & - C_5 \left( \frac{1}{2} \cosh(k\beta_1 L_2) k^2 \beta_1^2 - \frac{1}{2} \cos(k\beta_1 L_2) k^2 \beta_1^2 \right) \\ & - C_6 \left( -\frac{1}{2} \sin(k\beta_1 L_2) k^2 \beta_1^2 + \frac{1}{2} \sinh(k\beta_1 L_2) \right) k^2 \beta_1^2 = 0 \quad (16) \\ & C_3 \left( \frac{1}{2} \sinh(\beta_1 L_1) \beta_1^3 - \frac{1}{2} \sin(\beta_1 L_1) \right) \beta_1^3 + C_4 \left( \frac{1}{2} \cos(\beta_1 L_1) \beta_1^3 + \frac{1}{2} \cos h(\beta_1 L_1) \right) \beta_1^3 \\ & + C_5 \left( \frac{1}{2} \sinh(k\beta_1 L_2) k^3 \beta_1^3 + \frac{1}{2} \sin(k\beta_1 L_2) k^3 \beta_1^3 \right) \\ & + C_6 \left( -\frac{1}{2} \cos(k\beta_1 L_2) k^3 \beta_1^3 + \frac{1}{2} \cosh(k\beta_1 L_2) \right) k^3 \beta_1^3 = 0 \end{aligned}$$

Matrix A of the system (16) looks like:

$$A = \begin{bmatrix} \cosh(\beta_1 L_1) - \cos(\beta_1 L_1) & \sinh(\beta_1 L_1) - \sin(\beta_1 L_1) & -\sin(k\beta_1 L_2) - \sinh(k\beta_1 L_2) & -\cosh(k\beta_1 L_2) - \cos(k\beta_1 L_2) \\ \sinh(\beta_1 L_1) + \sin(\beta_1 L_1) & \cosh(\beta_1 L_1) - \cos(\beta_1 L_1) & k(\cos(k\beta_1 L_2) + \cosh(k\beta_1 L_2)) & k(-\sin(k\beta_1 L_2) + \sinh(k\beta_1 L_2)) \\ \cosh(\beta_1 L_1) + \cos(\beta_1 L_1) & \sinh(\beta_1 L_1) + \sin(\beta_1 L_1) & -k^2(-\sin(k\beta_1 L_2) + \sinh(k\beta_1 L_2)) & k^2(\cos(k\beta_1 L_2) - \cosh(k\beta_1 L_2)) \\ \sinh(\beta_1 L_1) - \sin(\beta_1 L_1) & \cosh(\beta_1 L_1) + \cos(\beta_1 L_1) & -k^3(\cos(k\beta_1 L_2) - \cosh(k\beta_1 L_2)) & k^3(\sinh(k\beta_1 L_2) + \sin(k\beta_1 L_2)) \end{bmatrix}$$

Equality to zero of the determinant  $D = \det(A) = 0$  of the matrix A is the equation aimed at finding natural frequencies.

$$\begin{aligned} & 4k(1 - \cosh(\beta_1 L_1) \sin(\beta_1 L_1) k^3 \cosh(k\beta_1 L_2) \sin(k\beta_1 L_2) - \\ & \cosh(\beta_1 L_1) \sin(\beta_1 L_1) k^3 \cos(k\beta_1 L_2) \sinh(k\beta_1 L_2) + \cosh(\beta_1 L_1) \cos(\beta_1 L_1) k^4 \cos(k\beta_1 L_2) \cosh(k\beta_1 L_2) + \\ & \cos(k\beta_1 L_2) \cosh(k\beta_1 L_2) + \sinh(\beta_1 L_1) \cos(\beta_1 L_1) k \cos(k\beta_1 L_2) \sin h(k\beta_1 L_2) - \\ & \sinh(\beta_1 L_1) \cos(\beta_1 L_1) k \cos h(k\beta_1 L_2) \sin(k\beta_1 L_2) - \\ & 2 \sinh(\beta_1 L_1) \sin(\beta_1 L_1) k^2 \sin(k\beta_1 L_2) \sinh(k\beta_1 L_2) + \\ & \cos(\beta_1 L_1) \sin h(\beta_1 L_1) k^3 \cos h(k\beta_1 L_2) \sinh(k\beta_1 L_2) + k^4 + \\ & \cosh(\beta_1 L_1) \cos(\beta_1 L_1) \cos(k\beta_1 L_2) \cosh(k\beta_1 L_2) + \sin(\beta_1 L_1) \cosh(\beta_1 L_1) k \cos(k\beta_1 L_2) \sin h(k\beta_1 L_2) - \\ & \sin(\beta_1 L_1) \cos h(\beta_1 L_1) k \cos(k\beta_1 L_2) \sin h(k\beta_1 L_2) + \\ & \cosh(\beta_1 L_1) \cos(\beta_1 L_1) - k^4 \cos(k\beta_1 L_2) \cosh(k\beta_1 L_2) - \cosh(\beta_1 L_2) \cos(\beta_1 L_2) k^4 = 0 \quad (17) \end{aligned}$$

In particular, with  $k = 0.1$ ,  $L_1 = 0.8 \text{ m}$ ,  $L_2 = 0.09 \text{ m}$ , the dependence of the normalized value of the determinant  $D(\beta_1)$  on the parameter  $\beta_1$  looks like (Figure 4)

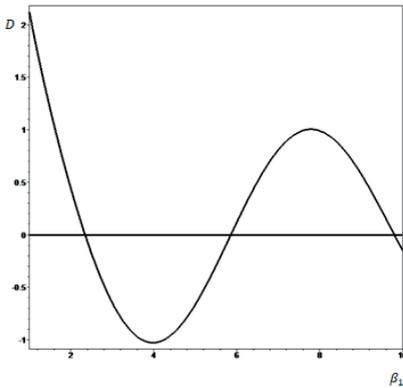


Figure 4. Plot of determinant versus parameter

The first three roots are equal, respectively:

$$\beta_{1_1} = 2.344, \beta_{1_2} = 5.868, \beta_{1_3} = 9.818$$

Analysis of the natural waveforms shows that the form corresponding to the second natural frequency is characterized by intensive movement of the part of the ear containing grains, which agrees with the results obtained earlier by the finite element method. The frequency interval corresponding to the second natural frequency can be called the low-frequency range, in which effective grain extraction from the ear is possible. It should be noted that this mode can be implemented directly in the field when the ear is not separated from the stem and the stem is not cut.

## RESULTS AND DISCUSSIONS

The second frequency range, in which effective grain extraction from the ear is possible, is the range of intense grain vibrations itself in the ear. Here, it should be noted, several modes associated with different grain maturity degrees, namely: the grain is attached to the stalk; the grain is held by the scales. This frequency range can be called - the high-frequency range, because it is a kilohertz interval.

To carry out numerical calculations on the developed mathematical model, experimental data on the deformation and destruction of

plant elements obtained earlier were used (Shack Yu. et al., 2013; Lachuga Y. et al., 2019b; Matrosov M. et al., 2020).

Numerical experiments to determine the grain motion characteristics in this range were carried out in the software complex of finite element analysis ACELAN (Belokon A. et al., 2000; Belokon A. et al., 2002). Quadratic finite elements were used, the finite-element mesh was thickened until the results of the calculations became independent of its shape.

In a numerical experiment the fluctuations of an individual grain at different stages of its maturation were considered: in case of its connection with the ear (early stage) and in the absence of such a connection (late stage).

Modal analysis was carried out, natural resonance frequencies and forms of fluctuations were found. Figures 5-19 show the results for the frequencies at which the fluctuations of the grain contribute to its separation from the ear.

The Figures 5-19 show the parameters of the corresponding value indicated in the figure caption: the blue color corresponds to a smaller value, the red one to a larger one. Figures 5-6 shows the units of measurement. The sides of 1 square are 1 mm. The scale is identical for Figures 5-19.

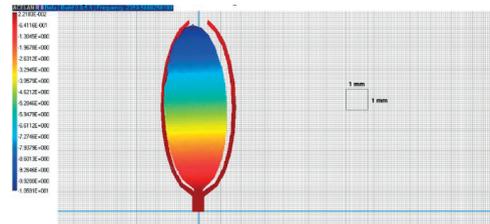


Figure 5. Bending form of grain fluctuation on the stem without scales' connection (horizontal displacement distribution) (fluctuation frequency 2.36 kHz)

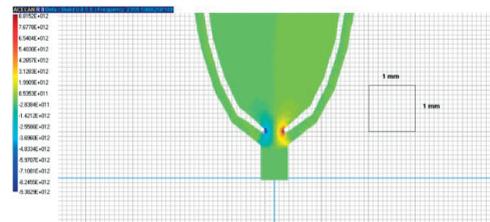


Figure 6. Bending form of grain fluctuation on the stem without scales' connection (distribution of normal vertical stresses) (fluctuation frequency 2.36 kHz)

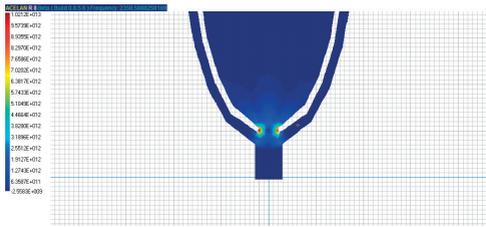


Figure 7. Bending form of grain fluctuations on the stem without scales' connection (stress intensity distribution) (fluctuation frequency 2.36 kHz)

Thus, Figures 5-7 show distributions of the stress-strain state (DSS) of the grain at its own frequency of 2.36 kHz, in the case of its not interacting with the scales, in the presence of connection of the grain with the ear through the stalk. The character of movement corresponds to the fluctuation of the grain at the stalk (Figure 5), in which the maximum stresses arise (Figures 6, 7), this may serve as the basis for its detachment from the ear. Figures 8 and 9 show similar results with a rigid connection between the grain and the flakes, due to their rigidity, the frequency increases compared to the previous case. There is a qualitative overlap of DSS characteristics with the previous case.

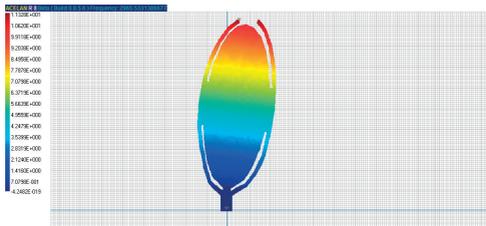


Figure 8. Bending form of grain fluctuation on the stem with a scales' connection (horizontal displacement distribution) (fluctuation frequency 2.97 kHz)

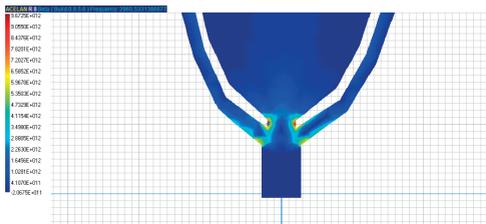


Figure 9. Bending form of grain fluctuation on the stem with scales' connection (stress intensity distribution) (fluctuation frequency 2.97 kHz)

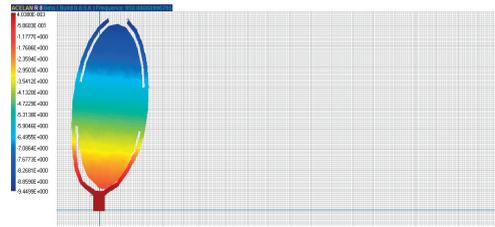


Figure 10. The bending form of grain fluctuation without a stalk with scales' connection (distribution of horizontal displacements) (fluctuation frequency 0.95 kHz)

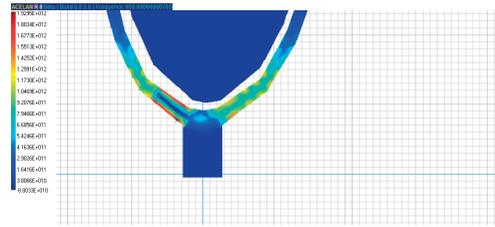


Figure 11. The bending form of grain fluctuations without a stem with scales' connection (stress intensity distribution) (fluctuation frequency 0.95 kHz)

Figures 10 and 11 show the results of bending grain fluctuations in the second maturity stage (no connection with the stalk), the maximum stresses occur at the base of the scales, which may be a sign of their destruction if the fluctuations amplitude is sufficient.

The figures below show fluctuations forms and characteristics of the stress-strain state during vertical movement of the grain without connection with the scales and in the presence of this connection. It should be noted, that the natural sequences in this case are much higher than the previous bending modes.

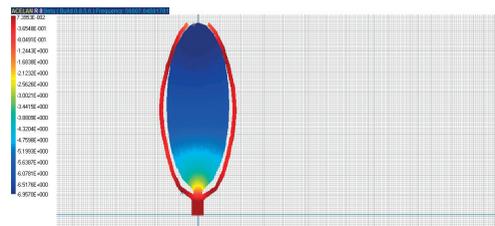


Figure 12. Vertical shape of grain fluctuation on the stem without scales' connection (distribution of vertical displacements) (fluctuation frequency 56.01 kHz)

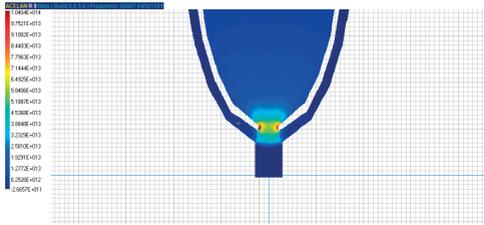


Figure 13. Vertical shape of grain fluctuation on the stem without scales' connection (distribution of stress intensity) (fluctuation frequency 56.01 kHz)

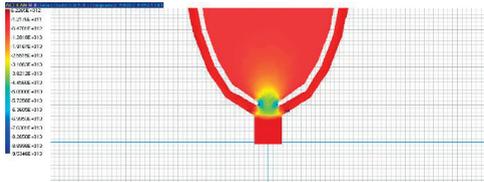


Figure 14. Vertical shape of grain fluctuation on the stem without scales' connection (vertical stress distribution) (fluctuation frequency 56.01 kHz)

Figures 12-14 show the distribution of vertical displacements and voltages at 56.01 kHz in the scale presence. The maximum stress level is in the material of the stalk, which contributes to its destruction. Figures 15-16 show similar results in the presence of a rigid connection to the scales. The natural frequency in this case is slightly lower and is 54.63 kHz. The maximum stress levels also, as in the previous case, occur in the stalk.

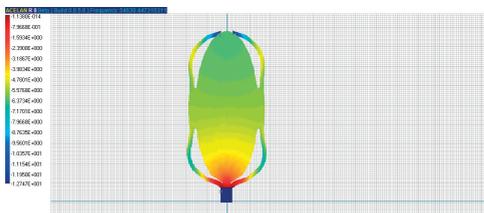


Figure 15. Vertical shape of grain fluctuation on the stem with scales' connection (distribution of vertical displacements) (fluctuation frequency 54.63 kHz)

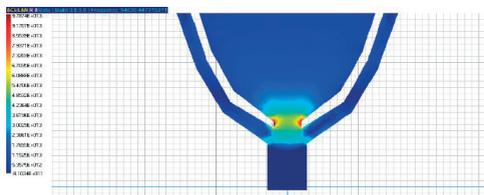


Figure 16. Vertical shape of grain fluctuation on the stem with scales' connection (distribution of stress intensity) (fluctuation frequency 54.63 kHz)

Finally, in this case, at the natural frequency of 61.88 kHz, intensive movement of the grain in the horizontal direction is observed in a fluctuation mode. Figure 17 shows the stress intensity distribution, the maximum of which is observed in the stalk, which contributes to its destruction.

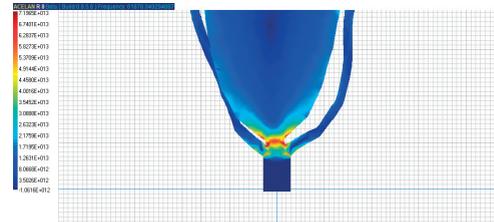


Figure 17. Shape of fluctuations with horizontal shift of the grain on the stem with scales' connection (distribution of stress intensity) (fluctuation frequency 61.88 kHz)

Figures 18-19 show vertical displacements and stress intensity distributions in the absence of the peduncle, but with the grain connection with the scales, at the natural frequency of 11.73 kHz, which, due to the lower rigidity of the scales compared to the peduncle, is much lower than the case in which there is a peduncle. The maximum stresses, as before, occur at the place where the scales are attached to the spike, which will also lead to their detachment.

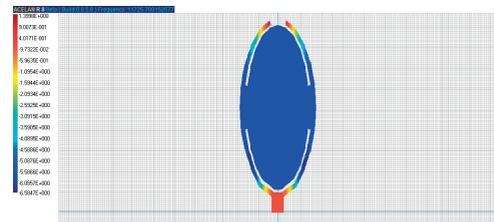


Figure 18. Vertical shape of the fluctuation of the grain without a stem with scales' connection (distribution of vertical displacements) (fluctuation frequency 11.73 kHz)

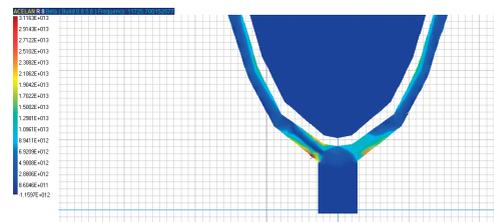


Figure 19. Vertical shape of grain fluctuations without a stem with scales' connection (distribution of stress intensity) (fluctuation frequency 11.73 kHz)

Analysis of the numerical results given in section 3 shows two frequency intervals of effective impact on the plants for the purpose of grain extraction.

The first low-frequency interval (tens of hertz, specific values depend on plant parameters) allows to excite significant ear vibrations, while the stem makes vibrations with relatively small amplitude. This frequency interval of influences can be used under field conditions, which will lead to active grains' extraction from the ear, leaving practically untouched stems.

The second interval is high-frequency (from tenths of kHz to tens of kHz, specific values depend on plant parameters and degree of grain ripening) characterized by intensive grain movement: these are bending grain movements with the stem and scales, which can lead to separation of the grain through breaking scales from the ear; and grain movement along the chamber, which can lead to its separation leaving scales undamaged, which is most beneficial from an energy consumption perspective.

## CONCLUSIONS

The article presents developed series of fluctuation models of the stem with the ear and the individual grain in the ear. The applied beam theory and the general continuum formulation within the linear theory of elasticity are used. Numerical calculations on the basis of the general model by means of the finite element method were performed. Numerical calculations of natural frequencies, fluctuation forms, and established fluctuations of the developed approaches are carried out both for the plant as a whole and its parts separately. The developed applied theories have shown their adequacy within the limits of their applicability.

Thus, models and tools for vibration analysis have been developed, allowing finding vibration amplitudes, mechanical stresses or forces (forces and moments) both analytically or numerically. These can be compared to critical values in plant elements destruction and grain extraction.

The natural frequencies show that at the value of natural frequencies of grain fluctuations

131,6 Hz and 1274,9 Hz the grain itself fluctuates predominantly, and at frequencies 1622,8 Hz, 7367.3 Hz, 9396.0 Hz and 13491,0 Hz the flakes vibrate.

Earlier field tests confirmed the results of theoretical and numerical analysis on the presence of two impact frequency ranges on the plant as a whole and on the ear, at which grains can be effectively extracted from the ear.

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## YIELD COMPONENTS OF TRITICALE AFTER APPLIED ELECTROMAGNETIC STIMULATION OF SEEDS

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### Abstract

*A field trial is conducted in Field Crops Institute (Bulgaria), in the period 2017-2019 on Pelic Verisols to evaluate the influence of three factors on yield triticale components: variety, electromagnetic seeds treatment and fertilization. Two Bulgarian varieties, two options of electromagnetic treatment and application of organic and mineral fertilizers are tested. The results show a positive effect of the electromagnetic treatment, the mineral and organic fertilizations. An increase the values of plant height, spike length, number of grains in a spike, grain weight and weigh of 1000 grains in the range of 0.4-6.6% for Colorit variety, and 1.5-9.2% for Boomerang variety is founded. Similar, significant effect of the fertilization on the spike length and the number of grains in a spike for Colorit variety, and for Boomerang variety both on the plant height and the grain weigh is established. No significant influence of the electromagnetic treatment and complex effect of the factors is reported. Based on the tested factors significant, positive correlations between different components of the yield for both the varieties are observed.*

**Key words:** *electromagnetic seeds treatment, mineral fertilizer, organic fertilizer, triticale, yield components.*

### INTRODUCTION

The yield of cereals is determined by a number of biometric and weight parameters, which change under the influence of agronomic factors, varietal specifics and environmental conditions, predominant during the growing season. The main components of the yield are the number of spikes per unit area, the number of grains in spike and the thousand kernel weight. There are close relationships between the components of yield, which under certain conditions determine their optimal development.

The main question concerning the yield of cultivated plants is how the components of the yield change, what connections and dependencies are observed between them against the background of specific agrotechnical approaches and agrometeorological conditions. By now, at cereals are defined quite regularities, both between yield and its components, and between the components, after testing various agrotechnical factors.

The results of Fischer (2011) show that the variation in a grain yield in wheat is closely related with changes in a number of grains in spike, than with average weight of the grain.

Pfeiffer et al. (1996) have founded when comparing wheat and triticale, that triticale forms a higher yield based of a larger number of grains per unit area and in class. Giunta and Motzo (2005) have reported that the higher yield of triticale compared to wheat, due to a larger number of spikes and grains per unit area. The comparative study of Méndez-Espinoza et al. (2019) found that the yield of triticale is explains by higher values of the thousand kernel weight and the number of grains in a spike and has a different physiological basis as higher photosynthetic rates. Bonchev (2020) has founded a high positive correlation of yield with plant height at varieties of common wheat.

Studies in cereals show that the components of the yield change to varying degrees under the influence of the applied factors. Biberdžić et al. (2010) have founded that the number of grains in a spike and the thousand kernel weight in triticale, show a different trend with increasing nitrogen fertilization and in barley they increase, respectively at 100 kg N/ha and 80 kg N/ha. Studies of Stefanova-Dobрева (2019) show that the length of spike, the number of grains in a spike and the weight of grains in a spike show a significant increase when

fertilization with  $N_6P_6$  and  $N_{12}P_6$ , depending of the variety.

Many scientists have established influence of environmental on the components of the yield. Đekić et al. (2016) have reported significant differences for the thousand kernel weight and the weight of grains in a spike in cereals in the years of study. Kendal & Sayar (2016) have established influence of environmental conditions on the height, the thousand kernel weight and the weight of grains in a triticale spike. The results obtained by Schwarte et al. (2006) show that the climatic conditions of the year affect the thousand kernel weight, the location of the experiment affects the number of grains in a spike, and the date of sowing affects the number of spike and tillers for unit area in triticale. Krusheva et al. (2018) have established that the climatic conditions of the year have the great influence on the number of grains in a spike and thousand kernel weight in triticale. In study by Hristov (2013) is founded that changes in the values of biometric indicators and the structural elements of triticale yield, follow those of yield, depending of the tillage systems tested and the fertilization options.

In modern agriculture is observed trend to apply intensive technological solutions, but it is interesting to follow the changes of the yield components when applying environmental approaches of impact. In research by Ivanova et al. (2008) soil pollutants are analyzed: chemical, heavy metals, pesticides, etc. and the advantages are indicated of the use of manure over chemical ones. The quality of the seeds is crucial for the amount of harvest obtained. Thus scientists looking for solutions for improvement of their sowing qualities. Now tests continue of different methods for pre-sowing treatment of seed material, one of them is exposing to an electromagnetic field. This ecological method allows to reduce the quantities of the mineral fertilizers used and successfully competes with the use of chemicals for disease control (Belitskaya et al., 2013).

It's known, that increase of yield is possible when applying of definite parameters of the electromagnetic field—voltage between the electrodes, seed exposure time, stay time of the seeds from treatment to sowing. Furthermore,

any agricultural crop has own optimum of these parameters, in which the yield is expected to increase. In Spain Flórez et al. (2014) and Martinez et al. (2017) have conducted a number of studies on pre-sowing magnetic treatment of triticale seeds. The parameters of the magnetic field are indicated, with which the seeds are treated. In Bulgaria has been studied and the positive impact has been established of electromagnetic seed pre-sowing treatments for triticale Muhova et al. (2016) on some laboratory parameters and on germination rate and mean germination time (Sirakov et al., 2016). Results for electromagnetic treatment of triticale seeds and their influence on the lengths and numbers of root and the lengths of the sprouts of the triticale Colorit variety, are also indicated by Sirakov et al. (2019).

The high productivity of the modern triticale varieties makes it preferred alternative culture. Due to high genetic yield potential, adaptability to environmental conditions and disease resistance, triticale is included to different systems of agriculture. The trend for the use of triticale is to be included as a useful ingredient in the food industry, respectively in the diet of humans. These benefits of triticale explain the wide interest to the culture and need to conduct of extensive research to increase yield.

The purpose of the research is to establish the effect of electromagnetic stimulation of triticale seeds on the components of yield, against the background of applied organic and mineral fertilization.

## MATERIALS AND METHODS

During the period 2017-2019 on conventional field, three-factor experiment was conducted by the method of perpendicular arrangement of degrees of the tested factors in four replications. The experimental plot was 18 m<sup>2</sup> in size and the sowing rate was 550 seeds per m<sup>2</sup>. The soil (PellicVertisols) is low to medium provided with mineral nitrogen, with low content of mobile phosphates and good supply of potassium. The sowing of triticale was carried out on 9.11.17 and 30.11.18 after the predecessor of sunflower. Before sowing phosphorus fertilizer and certified organic fertilizer were applied with subsequent cultivation in autumn. Then the soil was treated

with disc harrow. In the spring was imported nitrogen fertilizer. During the growing season

pest, weed and diseases were managed with insecticide, herbicides and fungicide.

Table 1. Temperature and precipitation conditions during the triticale vegetation

Years	Months									Σ
	X	XI	XII	I	II	III	IV	V	VI	
Temperature sums (°C) average for period 1928–2013										
<i>1928-13</i>	<i>396.7</i>	<i>215.9</i>	<i>61.1</i>	<i>-4.4</i>	<i>49.4</i>	<i>188.9</i>	<i>357.9</i>	<i>511.5</i>	<i>630.7</i>	<i>2137.7</i>
2017–2019										
2017/18	388.0	244.9	125.6	65.2	97.8	200.5	471.0	584.8	646.9	2827.7
2018/19	434.0	225.3	20.7	53.8	113.1	292.6	335.2	533.4	681.5	2689.6
Precipitation (mm) average for period 1928–2013										
<i>1928-13</i>	<i>37.5</i>	<i>43.3</i>	<i>54.0</i>	<i>44.3</i>	<i>37.7</i>	<i>37.0</i>	<i>45.2</i>	<i>64.1</i>	<i>65.4</i>	<i>428.5</i>
2017–2019										
2017/18	80.0	48.2	38.9	23.3	109.0	83.4	8.7	62.2	87.9	541.6
±	+42.5	+4.9	-15.1	-21.0	+71.3	+46.4	-36.5	-1.9	+22.5	+146.6
2018/19	25.4	82.3	46.9	28.9	24.5	3.3	51.4	21.4	123.2	383.9
±	-12.1	+39.0	-7.1	-15.4	-13.2	-33.7	+6.2	-42.7	+57.8	-41.1

Three factors were tested: two Bulgarian varieties of triticale–Boomerang and Colorit; organic certified fertilizer Lumbrical at a rates of 2,200 kg/ha and mineral fertilizers N<sub>120</sub>P<sub>60</sub>; pre-sowing electromagnetic treatment (EMT) with conditionally accepted options E0, E1 and E2. After preliminary laboratory test, were theoretically determined the values of the controllable factors of electromagnetic impact–voltage between the electrode space–U (kV), seed exposure time–τ (s), time from treatment to sowing T (days), in which it is expected increasing the seed germination and mean germination rate of seeds of both varieties, respectively expected increase in yield. The values of the controllable factors as follow: E1–U = 5.2 kV, τ = 24 s, T = 14 days; E2–U = 5.0 kV, τ = 50 s, T = 7 days. The option E0 is without electromagnetic influence. Plant samples (10 spikes) were collected from two plot at full maturity phase for processing.

To define significant influence of factors on data from the two harvest years 2018 and 2019, analysis of variance was applied separately for varieties with ANOVA (Penchev et al., 1989-1991). The coefficients of simple linear correlation of Pearson (r) were established with Statistika 13.

Table 1 shows the data from the meteorological station in Chirpan, regarding the temperature sum and the quantity of precipitation by months. According to Table 1, during the growing season of triticale the temperature

sums are higher compared to the multiannual period (2009.7°C), respectively for 2017/18 is 2824.7°C or 815.0°C more, and in 2018/19 is 2689.6°C or 679.9°C higher. Regarding precipitation: in 2017/18 the amount of precipitation is 146.6 mm more, and in 2018/19 is about average for the period 1928-2013 (395.0 mm) - 383.9 mm. This data characterize the two harvest years as warmer, and 2017/18 and as more humid, compared to typical weather conditions.

## RESULTS AND DISCUSSIONS

The results of analysis of variance for yield components for both varieties are shown on Table 2 and Table 3. The data from the statistical analysis for Colorit variety show no significant differences in the values for plant height (PH), length spike (LS), weight of grains in spike (WGS) and thousand kernel weight (TKW) (Table 2A). Highest values for LS, number of grains in spike (NGS) and WGS was reported for E1+N<sub>120</sub>P<sub>60</sub> option respectively, 5.6%, 16.6% and 5.7% more and for TKW for E2 option - 3.7% over the control option. The value for NGS is statistically significant at probability P = 5.0%. There is a trend to reduce PH values for E1 and E2 options compared to E0, but when applying mineral fertilizer the values increased by 10.5 and 10.2%, respectively.

Table 2. Influence of the factors on the components of yield for Colorit variety for 2017/19 period

## A. Influence of electromagnetic treatment and fertilization

Factors and levels	Plant height (cm)	Length spike (cm)	Number of grains in spike	Weight of grain in spike (g)	Thousand-kernel weight (g)
E0	100.8	10.8	62.0	2.63	39.96
E0+L	101.0 <sup>ns</sup>	10.6 <sup>ns</sup>	59.7 <sup>ns</sup>	2.41 <sup>ns</sup>	38.39 <sup>ns</sup>
E0+N <sub>120</sub> P <sub>60</sub>	106.7 <sup>ns</sup>	11.3 <sup>ns</sup>	69.3 <sup>ns</sup>	2.57 <sup>ns</sup>	37.05 <sup>ns</sup>
E1	97.5 <sup>ns</sup>	10.4 <sup>ns</sup>	62.4 <sup>ns</sup>	2.73 <sup>ns</sup>	41.30 <sup>ns</sup>
E1+L	97.2 <sup>ns</sup>	10.6 <sup>ns</sup>	65.6 <sup>ns</sup>	2.63 <sup>ns</sup>	39.41 <sup>ns</sup>
E1+N <sub>120</sub> P <sub>60</sub>	105.6 <sup>ns</sup>	11.4 <sup>ns</sup>	72.3*	2.78 <sup>ns</sup>	36.62 <sup>ns</sup>
E2	96.8 <sup>ns</sup>	10.8	63.7 <sup>ns</sup>	2.75 <sup>ns</sup>	41.42 <sup>ns</sup>
E2+L	97.8 <sup>ns</sup>	10.8	62.8 <sup>ns</sup>	2.71 <sup>ns</sup>	40.38 <sup>ns</sup>
E2+N <sub>120</sub> P <sub>60</sub>	102.5 <sup>ns</sup>	11.4 <sup>ns</sup>	66.1 <sup>ns</sup>	2.73 <sup>ns</sup>	40.47 <sup>ns</sup>
LSD (%)					
5.0	10.5	0.9	9.9	0.39	5.66
1.0	14.1	1.3	13.3	0.52	7.64
0.1	18.8	1.7	17.7	0.69	10.18

## B. Influence of electromagnetic treatment

Factors and levels	Plant height (cm)	Length spike (cm)	Number of grains in spike	Weight of grain in spike (g)	Thousand kernel weight (g)
E0	102.8	10.9	63.7	2.54	38.47
E1	100.1 <sup>ns</sup>	10.8 <sup>ns</sup>	66.4 <sup>ns</sup>	2.71 <sup>ns</sup>	39.11 <sup>ns</sup>
E2	99.0 <sup>ns</sup>	11.0 <sup>ns</sup>	64.2 <sup>ns</sup>	2.73 <sup>ns</sup>	40.75 <sup>ns</sup>
LSD (%)					
5.0	6.0	0.5	5.7	0.22	3.27
1.0	8.2	0.7	7.7	0.30	4.41
0.1	10.9	1.0	10.2	0.40	5.87

## C. Influence of fertilization

Factors and levels	Plant height (cm)	Length spike (cm)	Number of grains in spike	Weight of grain in spike (g)	Thousand kernel weight (g)
0	98.3	10.7	62.4	2.70	40.89
L	98.7 <sup>ns</sup>	10.7	62.7 <sup>ns</sup>	2.58 <sup>ns</sup>	39.39 <sup>ns</sup>
N <sub>120</sub> P <sub>60</sub>	104.9*	11.4*	69.2*	2.69 <sup>ns</sup>	38.05 <sup>ns</sup>
LSD (%)					
5.0	6.0	0.5	5.7	0.22	3.27
1.0	8.2	0.7	7.7	0.30	4.41
0.1	10.9	1.0	10.2	0.40	5.87

\*significant at probability P = 0.1%; ns - no significant; L - Lumbrical; LSD - low significant differences.

Although, that the values of all tested components were statistically no significant under the influence of the EMT, from the results of Table 2B it can be found that for E1 and E2 options the PH values were lower than E0, and for WGS and TKW higher for E2 option by 7.5% and 5.9%, respectively compared to E0. The results for LS and NGS were highest for E2 and E1 options, respectively 0.9% and 4.7% compared to E0. According to Table 3C, mineral fertilization has statistically significant influence for the PH, LS and NGS - 5.6%, 6.5% and 10.8%, respectively compared to the control option. They were not established significant differences for WGS and TKW. Higher, but no

significant values for PH and NGS after application of organic fertilizer are reported - 0.4 and 1.0%, respectively compared to the control option.

In contrast from Colorit variety, yield components under the influence of EMT and fertilization for Boomerang variety showed highest values for different options of EMT and fertilization (Table 3A). The values for PH and WGS showed highest results for E2+N<sub>120</sub>P<sub>60</sub> option - 7.0% and 8.5% above E0, respectively. The PH is significant at probability P = 5.0%. The value of LS was influenced most favourably for E0+N<sub>120</sub>P<sub>60</sub> - 3.6%, and NGS for E1+N<sub>120</sub>P<sub>60</sub> option - 9.2% above E0. WGS showed highest, but no significant value for E2

Table 3 Influence of the factors on the yield components for Boomerang variety for 2017/19 period

## A. Influence of electromagnetic treatment and fertilization

Factors and levels	Plant height (cm)	Length spike (cm)	Number of grains in spike	Weight of grain in spike (g)	Thousand-kernel weight (g)
E0	108.0	11.1	54.6	2.58	45.88
E0+L	108.4 <sup>ns</sup>	10.4 <sup>ns</sup>	55.0 <sup>ns</sup>	2.51 <sup>ns</sup>	43.94 <sup>ns</sup>
E0+N <sub>120</sub> P <sub>60</sub>	113.0 <sup>ns</sup>	11.5 <sup>ns</sup>	60.3 <sup>ns</sup>	2.63 <sup>ns</sup>	43.43 <sup>ns</sup>
E1	106.1 <sup>ns</sup>	10.8 <sup>ns</sup>	55.4 <sup>ns</sup>	2.49 <sup>ns</sup>	43.81 <sup>ns</sup>
E1+L	108.2 <sup>ns</sup>	10.5 <sup>ns</sup>	50.8 <sup>ns</sup>	2.34 <sup>ns</sup>	42.78 <sup>ns</sup>
E1+N <sub>120</sub> P <sub>60</sub>	113.2 <sup>ns</sup>	11.2 <sup>ns</sup>	59.6 <sup>ns</sup>	2.72 <sup>ns</sup>	43.47 <sup>ns</sup>
E2	105.4 <sup>ns</sup>	10.4 <sup>ns</sup>	56.5 <sup>ns</sup>	2.63 <sup>ns</sup>	47.39 <sup>ns</sup>
E2+L	107.8 <sup>ns</sup>	10.3 <sup>ns</sup>	50.2 <sup>ns</sup>	2.52 <sup>ns</sup>	44.56 <sup>ns</sup>
E2+N <sub>120</sub> P <sub>60</sub>	115.6 <sup>*</sup>	11.3 <sup>ns</sup>	59.5 <sup>ns</sup>	2.80 <sup>ns</sup>	45.32 <sup>ns</sup>
LSD (%)					
5.0	7.4	11.0	9.2	0.35	3.63
1.0	10.0	10.8 <sup>ns</sup>	12.5	0.47	4.91
0.1	13.4	10.6 <sup>ns</sup>	16.6	0.63	6.53

## B. Influence of electromagnetic treatment

Factors and levels	Plant height (cm)	Length spike (cm)	Number of grains in spike	Weight of grain in spike (g)	Thousand-kernel weight (g)
E0	109.8	11.0	56.6	2.57	44.36
E1	109.1 <sup>ns</sup>	10.8 <sup>ns</sup>	55.3 <sup>ns</sup>	2.52 <sup>ns</sup>	43.85 <sup>ns</sup>
E2	109.6 <sup>ns</sup>	10.6 <sup>ns</sup>	55.4 <sup>ns</sup>	2.65 <sup>ns</sup>	45.75 <sup>ns</sup>
LSD (%)					
5.0	4.3	0.6	5.3	0.20	2.10
1.0	5.8	0.8	7.2	0.27	2.83
0.1	7.7	1.0	9.6	0.36	3.77

## C. Influence of fertilization

Factors and levels	Plant height (cm)	Length spike (cm)	Number of grains in spike	Weight of grain in spike (g)	Thousand-kernel weight (g)
0	106.5	10.8	55.5	2.57	45.69
L	108.1 <sup>ns</sup>	10.4 <sup>ns</sup>	52.0 <sup>ns</sup>	2.45 <sup>ns</sup>	44.26 <sup>ns</sup>
N <sub>120</sub> P <sub>60</sub>	113.9 <sup>**</sup>	11.3 <sup>*</sup>	59.8 <sup>ns</sup>	2.72 <sup>ns</sup>	44.01 <sup>ns</sup>
LSD (%)					
5.0	4.3	0.6	5.3	0.20	2.10
1.0	5.8	0.8	7.2	0.27	2.83
0.1	7.7	1.0	9.6	0.36	3.77

\* \*\*significant at probability P = 0.1 and P = 1.0%; ns - no significant; L - Lumbrical; LSD - low significant differences.

+N<sub>120</sub>P<sub>60</sub> option - 8.5% more. Like of Colorit variety TKW was highest, but no significant for E2 option - 3.3% to E0.

According to Table 3B, EMT does not influence significant on the values of the studied components. The results for WGS and TKW were highest for E2 option - 3.1% to E0. It is observed reduction of the values of LS and NGS under the influence of E1 and E2 options compared to E0.

The results of the effect of fertilization (Table 3C) showed that mineral fertilization has statistically significant effect for PH and LS, the values are 6.9% and 5.6% more than control option. It is reported increase the value of PH by 1.5%, after application of organic fertilizer. From the results presented on Table 2B and Table 3B should be summarized, that

for both triticale varieties the values for WGS and TKW were highest for E2 treatment. This means, that the EMT has a positive impact on these indicators, which mainly express spike productivity in cereals. In study by Belitskaya et al. (2013) also is established, that under the influence of electromagnetic stimulation increased field germination is obtained, it is improved the initial development of root, length of sprouts, the yield components for triticale - PH, WGS and TKW exceed the control. In study by Tibirkov et al. (2012) is established, that TKW has highest value after EMT in wheat seeds, compared to the control option. Different results have received Zhalnin et al. (2016). Their research establishes no significant influence of electromagnetic field on TKW in spring wheat.

Table 4. Effect of electromagnetic treatment and fertilization on the plant height

Source of variation	df	SS	$\eta$ (%)	MS	F criteria	P value
Colorit						
Options	8	440.5313	23.88	55.06641	1.058545	0.42001
A	2	92.59375	5.0	46.29688 <sup>ns</sup>	0.889968	0.57476
B	2	332.5938	18.0	166.2969 <sup>ns</sup>	3.196736	0.05542
A×B	4	15.34375	0.8	3.835938 <sup>ns</sup>	7.373849E-02	0.98663
Error	27	1404.563	76.1	52.02083	-	-
Boomerang						
Options	8	401.0938	36.15086	50.13672	1.910897	0.09949
A	2	2.75	0.2	1.375 <sup>ns</sup>	5.240637E-02	0.94898
B	2	369.875	33.2	184.4375**	7.0296	0.00378
A×B	4	29.46875	2.7	7.367188 <sup>ns</sup>	0.280791	0.88738
Error	27	708.4063	63.8	26.23727	-	-

\*\*significant at probability P = 1.0%; ns - no significant; A - electromagnetic treatment; B - fertilization.

Table 5. Effect of electromagnetic treatment and fertilization on the length of spike

Source of variation	df	SS	$\eta$ (%)	MS	F criteria	P value
Colorit						
Options	8	5.038574	31.26	0.6298218 <sup>ns</sup>	1.534754	0.19151
A	2	0.2802735	1.74	0.1401367 <sup>ns</sup>	0.341486	0.71834
B	2	4.350586	27.00	2.175293*	5.300767	0.01136
A×B	4	0.4077149	2.53	0.1019287 <sup>ns</sup>	0.2483805	0.90712
Error	27	11.08008	68.74	0.4103733	-	-
Boomerang						
Options	8	6.601563	33.85	0.8251953 <sup>ns</sup>	1.726839	0.13736
A	2	0.7084961	3.63	0.3542481 <sup>ns</sup>	0.7413147	0.51000
B	2	5.283692	27.10	2.641846**	5.52844	0.00973
A×B	4	0.609375	3.12	0.1523438 <sup>ns</sup>	0.3188011	0.86307
Error	27	12.90234	66.15	0.4778646	-	-

\*,\*\*significant, respectively at probability P = 0.1 and P = 1.0%; ns - no significant; A - electromagnetic treatment; B - fertilization.

Table 6. Effect of electromagnetic treatment and fertilization on the number of grains in spike

Source of variation	df	SS	$\eta$ (%)	MS	F criteria	P value
Colorit						
Options	8	492.9531	28.31	61.61914 <sup>ns</sup>	1.332923	0.26951
A	2	65.85938	3.78	32.92969 <sup>ns</sup>	0.712323	0.50362
B	2	340.625	19.56	170.3225*	3.684138	0.03748
A×B	4	86.46875	4.97	21.61719 <sup>ns</sup>	0.467151	0.76120
Error	27	1248.172	71.67	46.22859	-	-
Boomerang						
Options	8	368.211	25.20	46.02637	1.136795	0.37139
A	2	2.242188	0.15	1.121094	2.768964e-02	0.97339
B	2	300.336	20.55	150.168*	3.708964	0.03675
A×B	4	65.63281	4.49	16.4082	0.4052625	0.80470
Error	27	1093.172	74.80	40.48785	-	-

\*significant at probability P = 0.1%; ns - no significant; A - electromagnetic treatment; B - fertilization.

On the other components of the yield for both triticale varieties, different effects of EMT options were observed. Nizharadze (2016) has received similar results.

The analysis of variance showed significant effect of fertilization for PH, LS, NGS and WGS for Boomerang variety (Tables 4, 5, 6 and 7). No significant effects of

Table 7. Effect of electromagnetic treatment and fertilization on the weight of grain in spike

Source of variation	df	SS	$\eta$ (%)	MS	F criteria	P value
Colorit						
Options	8	0.4247742	18.19	5.309677e-02	0.7501617	0.64904
A	2	0.2706909	11.59	0.1353455	1.912188	0.16565
B	2	0.1082764	4.64	5.413819e-02	0.764875	0.52084
A×B	4	4.580689e-02	1.96	1.145172e-02	0.1617922	0.95349
Error	27	1.911072	81.81	7.078043e-02		
Boomerang						
Options	8	0.6035461	28.00	7.544327e-02	1.312917	0.27864
A	2	0.1078339	5.00	5.391693e-02	0.9383003	0.59394
B	2	0.4190674	19.44	0.2095337*	3.646453	0.03861
A×B	4	0.0766449	3.56	1.916122e-02	0.3334571	0.85343
Error	27	1.551483	72.00	5.746234e-02	-	-

\*significant at probability P =0.1%; ns - no significant; A - electromagnetic treatment; B - fertilization.

Table 8. Effect of electromagnetic treatment and fertilization on the thousand kernel weight

Source of variation	df	SS	$\eta$ (%)	MS	F criteria	P value
Colorit						
Options	8	97.41797	19.17	12.17725	0.8006728	0.60821
A	2	33.44922	6.58	16.72461	1.099669	0.34834
B	2	48.58985	9.56	24.29492	1.597429	0.21972
A×B	4	15.37891	3.03	3.844727	0.2527968	0.90449
Error	27	410.6367	80.8253	15.20877	-	-
Boomerang						
Options	8	56.63281	25.07	7.079102	1.129206	0.37591
A	2	23.28125	10.31	11.64063	1.856826	0.17401
B	2	19.78906	8.76	9.894531	1.578302	0.22357
A×B	4	13.5625	6.00	3.390625	0.5408475	0.70980
Error	27	169.2656	74.93	6.269098	-	-

ns - no significant; A - electromagnetic treatment; B - fertilization.

fertilization and EMT were founded on the thousand kernel weight (Table 8). It was established close effect of the fertilization on both varieties of triticale, respectively 27.0 and 27.1% of the variance for LS and 19.5 and 20.6% for NGS. The greatest effect of fertilization was reported on the PH for Boomerang variety - 33.2% of the total variation. For yield components for both varieties no significant effect of EMT was found. This gives basis to note that the significant values to E0 for NGS and PH, shown in Table 2A and Table 3A, are due to of the positive influence of mineral fertilization. From Table 9 can to be fined, that in both varieties of triticale the established correlation coefficients show linear, positive relationships between some of the components studied, but differences are observed. For Colorit variety can to affirm, that there are statistically significant dependencies between all

investigated components. High correlations were found between NGS and WGS ( $r = 0.775$ ), between WGS and TKW ( $r = 0.869$ ). For Boomerang variety there are no correlations between LS and the other components, PH low correlated with WGS ( $r = 0.335$ ) and LS ( $r = 0.454$ ).

## CONCLUSIONS

It is established similar, significant effects of fertilization on the length of a spike and the number of grains in a spike for both varieties of triticale, and for Boomerang variety on the plants height and the weight of the grains in spike. Not confirmed the effect of electromagnetic pre-sowing seed treatment, but is reported positive impact on some components of the yield, expressed with higher values compared to the control option.

Table 9. Correlation coefficients between the components of grain yield

	PH	SL	NGS	WGS	TKW
Colorit					
PH	1				
LS	0.631***	1			
NGS	0.552***	0.674***	1		
WGS	0.446***	0.691***	0.775***	1	
TKW	0.334*	0.564***	0.426***	0.869***	1
Boomerang					
PH	1				
LS	0.454***	1			
NGS	0.260	0.265	1		
WGS	0.335*	0.187	0.855***	1	
TKW	0.1456	0.049	0.534***	0.688***	1

\*, \*\*\*, significant, respectively at probability,  $\alpha = 0.05$  and  $\alpha = 0.01$ ; n = 35 observations; PH - Plant height; SL - Spike length; NGS - Number of grain per spike; WGS - Weight of grains per spike; TKW - Thousand kernel weight.

For Colorit variety is received an increase for the following yield components: for the length of a spike, the number of grains in a spike and the weight of the grains in a spike for E1 option and applied mineral fertilizer N<sub>120</sub>P<sub>60</sub>, respectively 5.6, 16.6 and 5.7%; for the length of a spike, the weight of the grains in a spike and the thousand kernel weight at separately application of E2 option, respectively 0.9, 7.5 and 5.9%; for the number of grains in a spike for E1 option—4.7%.

For Boomerang variety highest values are received for the following yield components: for the plant height and the weight of the grains in a spike for E2 option and applied mineral fertilizer N<sub>120</sub>P<sub>60</sub> - 7.0 and 8.5%; for the weight of the grains in a spike and the thousand kernel weight when applying separately E2 option - 3.1%.

When applying organic fertilizer in both varieties of triticale, the increase in values of some components of the yield is within 0.4 and 1.5%, and after mineral fertilization from 5.6 to 10.8%.

Positive correlations are established between all yield components for the variety Colorit, and for Boomerang variety between the plant height and the length of a spike, as well as interdependence between the number of grains in a spike, the weight of the grains in a spike and the thousand kernel weight.

Pre-sowing electromagnetic treatment is a promising ecological method for stimulating effect on triticale seeds. It is necessary

conducting field experiments with electromagnetically treated seeds.

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## THE EFFECTIVENESS OF SOME INSECTICIDE TREATMENTS AGAINST *Tanymecus dilaticollis* Gyll. AT MAIZE

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### Abstract

In Romania, maize ranks the first place regarding the cultivated area. In 2019, there were cultivated over 2.68 million ha with maize, with a total production of 17.43 million tonnes. The maize yield is influenced by the climatic conditions, applied crop technology, but also by the pest attack which can have a significant effect on the final yield.

The objective of the present paper is to present the effectiveness of different pesticide treatments (seed and vegetation treatments) against the maize leaf weevil (*Tanymecus dilaticollis* Gyll.) attack in the maize crops from South Romania. The researches were performed under field conditions in South Romania (Giurgiu county, Putineiu location) in 2019. The experimental factors were the followings: Factor A: pesticide seed treatment, with three variants (no pesticide seed treatment; Nuprid AL 600 FS (600 g/l imidacloprid), in dose of 8 lt of seeds; Actara 25 WG (25% thiametoxam), in dose of 10 kg/t of seeds); Factor B: pesticide treatment in the vegetation period, after plant emergence, with three variants (no pesticide treatment; Mospilan 20 SG (200 g/kg acetamiprid), in dose of 0.1 kg/ha; Actara 25 WG (25% thiametoxam), in dose of 0.1 kg/ha; Lamdex Extra (25 g/kg lambda-cihalotrin), in dose of 0.3 kg/ha); Factor C: maize hybrid, with two variants (Olt hybrid; DKC4351 hybrid).

The higher insecticide effectiveness in terms of productivity was registered in the variant with the seed treatment with Nuprid AL 600 FS, with 12,460 kg/ha and with a plant density of 70,536 plants/ha. The highest maize leaf weevil attack was recorded on the control variant with no pesticide treatment applied (no pesticide seed treatment and no insecticide applied in the vegetation period). In the case of spraying insecticides in the first vegetation stages against maize leaf weevil, the higher effectiveness was recorded on the variant treated with Mospilan 20 SG.

**Key words:** maize, *Tanymecus dilaticollis*, insecticides, seed treatment, crop protection.

### INTRODUCTION

Maize (*Zea mays* L.) is one of the most important crop at world level. This ranks the second place as harvested area (197.2 million ha, according to FAO data) after wheat, this being due to its high capacity of production, to its wide ecological plasticity which gives it the ability to be sown on a large types of agricultural areas.

From a technological point of view, it can be resown on the same area in a monoculture system, it's a fully mechanised crop and it put into value very well the organically and mineral fertilisers.

Besides these arguments, maize is also cultivated because of its variety of possibilities to be used.

In Romania, maize ranks the first place regarding the cultivated area. In 2019, there were cultivated over 2.68 million ha with

maize, with a total production of 17.43 million tons (FAOSTAT, 2019).

The maize yield is influenced by the climatic conditions, the applied crop technology, but also by the pest attack which can have a significant effect on the final yield.

Regarding the pest attack on maize, one of its most important pests which makes a lot of damages on the crop, even compromising the crop, is the maize leaf weevil (*Tanymecus dilaticollis* Gyll.) (Barbulescu, 1977). Adults of maize leaf weevil attack the young plants in course of and after emergence, producing total damage on their leaves (Paulian et al., 1979; Voinescu, 1985). If the pest is not controlled in proper time, farmers have to resowing the crop because it is compromised (Barbulescu, 2001). Maize leaf weevil is considered the most harmful pest on maize crops on European level, a majority of its attack being identified in the South-Eastern area of the continent (Paulian, 1972).

In Bulgaria, the *T. dilaticollis* attack is mostly identified on maize, sunflower and beetroot crops (Kirkov, 1967; Krusteva et al., 2006). Also, significant damages are recorded in Eastern Croatia (Čamprag et al., 1969). Researches conducted in 2010 in Greece in the maize areas put into evidence the aggressive attack of the maize leaf weevil. Even though plants emergence is successfully they were damaged almost completely (Papadopoulou, 2012).

Maize leaf weevil was identified also in Ukraine, where it caused major damage on maize and beetroot crops (Dieckmann, 1983).

In Romania, maize leaf weevil is mostly found in the South-Eastern area of the country, this area being very favourable for pest growth (Paulian, 1972). Researches shown over time that the attack is a very intense one, with high density of pest population when facing monoculture system (Paulian, 1972; Voinescu and Barbulescu, 1998; Popov & Barbulescu, 2007; Georgescu et al., 2014).

Regarding pest controlling measures, best results were recorded when using systemic insecticide treatments on the seeds, which ensure plant protection in the first growing stages. Best results against maize leaf weevil are given by active substances from neonicotinoids class. If the seeds treatment was not properly conducted, one can apply a contact insecticide in the first growing stages. But, this is supposed to be more a correction treatment.

Over the years, in Romania, there were a lot of discussions about using the neonicotinoid class active substances. In 2013, following the 485<sup>th</sup> directive of the European Commission, three of the neonicotinoids substances were banned: imidacloprid, thiametoxam, and clotianidin, which left the farmers without any approved substance for seeds treatment against maize leaf weevil. Currently, waivers for using these substances are obtained annually, but only for maize seeds treatment. This leads to sunflower crops still remaining without any approved seeds treatment against maize leaf weevil.

The objective of the present paper is to present the effectiveness of different pesticide treatments (seed and vegetation treatments) against the maize leaf weevil attack in the maize crops from South Romania.

## MATERIALS AND METHODS

A field experience was conducted in the Southern area of the country, in the Burnaz Plain, in Giurgiu county, Putineiu location (43°52'59" North Latitude, 25°40'1" East Longitude, 67 m altitude), in the year 2019. The experimental factors were the followings:

- Factor A: pesticide seed treatment, with three variants, respectively:
  - No pesticide seed treatment;
  - Nuprid AL 600 FS (600 g/l imidacloprid), in dose of 8 l/t of seeds;
  - Actara 25 WG (25% thiametoxam), in dose of 10 kg/t of seeds.
- Factor B: pesticide treatment in the vegetation period, after plant emergence, with three variants, respectively:
  - No pesticide treatment;
  - Mospilan 20 SG (200 g/kg acetamiprid), in dose of 0.1 kg/ha;
  - Actara 25 WG (25% thiametoxam), in dose of 0.1 kg/ha;
  - Lamdex EXTRA (25 g/kg lambda-cihalotrin), in dose of 0.3 kg/ha.
- Factor C: maize hybrid, with two variants, respectively:
  - Olt hybrid;
  - DKC4351 hybrid.

Experimental plots had a length of 40 m, with 4.2 m width (6 plant rows), resuming in a total surface of 4000 m<sup>2</sup>.

The preceding plant was winter wheat. The sowing was performed on 10<sup>th</sup> of April with a tractor and the SPC 6 seeder, the distance between rows being of 0.7 m and between the maize seeds being of 0.19 m. The planned density was of 75,000 seeds/ha.

From each experimental plot, 40 plants from 4 rows have been assessed. 10 plants per each row have been marked with stakes, marked plants being assessed in a "stairs" system. The attack intensity of the *T. dilaticollis* was assessed when plants arrived at two leaf stage (BBCH 12), using a scale from 1 to 9, elaborated and improved by Paulian (1972), where 1 means plant is not attacked and 9 means plant is destroyed completely, with leaves chafed close to soil level.

After the assessment of the attack intensity, then the vegetation treatments were applied. After another 15 days, measurements were

made on the number of plants saved after the vegetation treatments. They consisted in assessing the new damages registered on the plants, without taking into account the damages discovered when the intensity of the attack before applying the vegetation treatments was measured.

In the spring of 2019, the climatic conditions were optimal for maize crop, but in the same time very favourable for the pests' attacks. The medium temperature in April was of 14°C. On the sowing date, 14°C were registered and the soil temperature was of 9°C, with a growing tendency. Regarding the rainfalls, two days after sowing 35 mm rainfall were registered, which lead to maize germinating and rising in optimal parameters.

The field experience was performed on a chernozem soil type, with a high content of humus (over 4%), with a slightly acid pH (6.4), well supplied with nutrients and very good water and aeration properties.

## RESULTS AND DISCUSSIONS

Regarding the intensity of *T. dilaticollis* attack before the vegetation treatment was applied, it is noticed that the highest value (a medium note of 4.75) was registered on the experimental plot sown with Olt hybrid, without seed treatments. The lowest one can be observed on the experimental plot sown with Olt hybrid, with Actara treated seeds (a medium note of 3.97) (Figure 1).

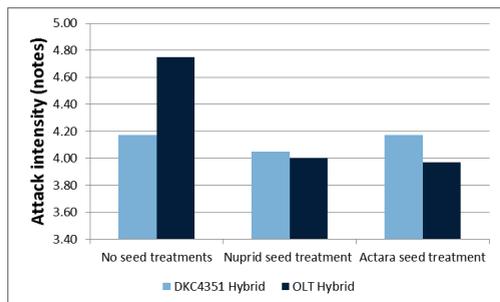


Figure 1. Intensity of *T. dilaticollis* attack before the vegetation treatment

Concerning the intensity of *T. dilaticollis* attack after the vegetation treatment was applied, it is noticed that the highest value (a medium note of 4.42) was registered on the experimental plot

sown with DKC 4351 hybrid, with Actara treated seeds and no vegetation treatments. The lowest one can be observed on the experimental plot sown with DKC 4351 hybrid, with Nuprid treated seeds and both Mospilan and Actara treatments applied in vegetation (both plots with a medium note of 3.22) (Figure 2).

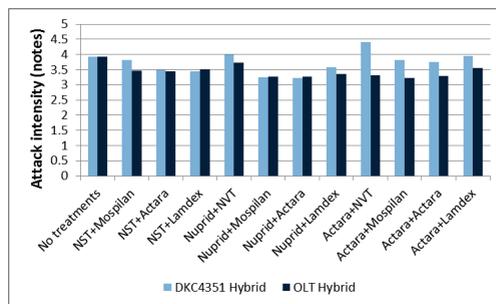


Figure 2. Intensity of *T. dilaticollis* attack after the vegetation treatment

\*Legend: NST - no seeds treatment; NVT - no vegetation treatment

Regarding the density of plants on hectare, the experimental plot sown with Olt hybrid stands out, with Nuprid treated seeds and Mospilan treatment applied in vegetation (density of 74,196 plants/ha). The lowest density was recorded on the experimental plot sown with DKC 4351 hybrid with Actara treatments applied both on seeds and in vegetation (density of 49,286 plants/ha) (Figure 3).

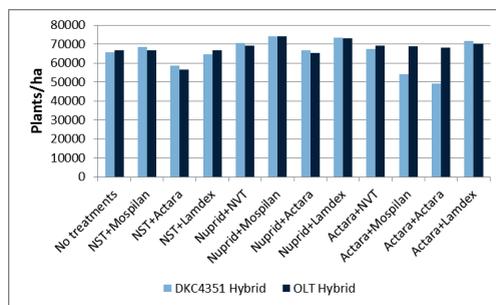


Figure 3. Density of plants depending on experimental plot

\*Legend: NST - No seeds treatment; NVT - No vegetation treatment

Regarding the grain yield obtained on hectare, the experimental plot sown with DKC 4351 hybrid stands out, with Nuprid treated seeds and no treatments applied in vegetation (production of 12,460 kg/ha). The lowest grain yield was recorded on the experimental plot

sown with Olt hybrid with no treatment applied on seeds and Actara treatments applied in vegetation (production of 7716 kg/ha) (Figure 4).

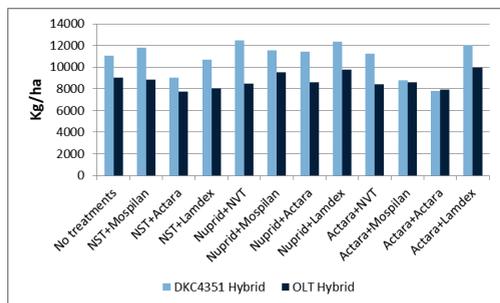


Figure 4. Grain yield recorded depending on experimental plot

\*Legend: NST - No seeds treatment; NVT - No vegetation treatment

## CONCLUSIONS

Before the vegetation treatment was applied, the most damaging *T. dilaticollis* attack was recorded on the experimental plots without seeds treatments, especially in the case of the Olt hybrid.

After the vegetation treatment was applied, the most damaging *T. dilaticollis* attack was registered on the experimental plot sown with DKC 4351 hybrid, with Actara treated seeds and no vegetation treatments.

The highest plant density on hectare was recorded on the experimental plot sown with Olt hybrid, with Nuprid treated seeds and Mospilan treatment applied in vegetation.

The highest grain yield obtained on hectare was recorded on the experimental plot sown with DKC 4351 hybrid, with Nuprid treated seeds and no treatments applied in vegetation.

Currently, vegetation treatments can be considered an alternative in the fight with the maize leaf weevil but an expensive one and with a significant smaller yield than seeds treatments. The main method of controlling *T. dilaticollis* will still remain a properly performed seeds treatment with a high efficacy insecticide.

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## RESPONSE OF BREAD WHEAT (*Triticum aestivum* L.) VARIETIES TO NITROGEN FERTILIZER RATES AT MEKDELA DISTRICT, SOUTH WOLLO, ETHIOPIA

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### Abstract

This study was initiated to assess the effect of N fertilizer rates on growth, yield, and quality of newly released bread wheat (*Triticum aestivum* L.) varieties and identify the optimum N fertilizer rates and appropriate bread wheat variety. A field experiment was carried out in 2019 main cropping season at Mekdela, South Wollo, Ethiopia. Factorial combinations of two wheat cultivars Lemu (ETBW 6861) and Wane (ETBW 6130) and five rates of N (0, 46, 92, 138 and 184 kg N ha<sup>-1</sup>), were laid out in a Randomized Complete Block Design with three replications. Pre planting soil analyses results revealed that soil textural class was clay, medium in total N (0.2%), slightly acidic reaction and medium in organic matter (4.3%). Though the interaction of N rate and variety exhibited fewer effects, the soil analyses after harvest showed that N rate had residual effect on the soil. The variety Lemu had higher values for effective tillers, spike length, plant height, number of seeds spike<sup>-1</sup>, grain and straw yield, hectoliter weight and grain N uptake. On the other hand, Wane variety exhibited greater values for thousand seed weight, dry and wet gluten, and with short phenological period. Although the difference was non-significant in HI, straw and total N uptake agronomic and physiological efficiency and grain protein content between varieties, Wane had greater agronomic and physiological efficiency, straw N uptake and HI. However, Lemu variety had higher total N uptake and protein content than Wane variety. Days to maturity, effective tillers, plant height and straw yield were consistently increased in response to N rates. The highest values of seed spike<sup>-1</sup> (57.4), thousand seed weight (44.5 g), grain yield (7415 kg ha<sup>-1</sup>), HI (36.3%), hectoliter weight (82.53 kg hl<sup>-1</sup>) and physiological efficiency (45.48 kg kg<sup>-1</sup>) were recorded at 92 kg N ha<sup>-1</sup>; whereas the highest agronomic efficiency (48.65 kg kg<sup>-1</sup>) was obtained from 46 kg N ha<sup>-1</sup>. Though inconsistent increments of grain and straw N uptake, dry and wet gluten increased with increased N rates, the highest values recorded from 184 kg N ha<sup>-1</sup>. The longest spike length was obtained from 92 kg N ha<sup>-1</sup> for Lemu (9.33 cm) and 138 kg ha<sup>-1</sup> for Wane (7.13 cm). The longest days to grain filling and highest grain protein was recorded from 184 kg ha<sup>-1</sup> for both varieties. The partial budget analysis results revealed that the highest marginal rate of return was 980% at 92 kg N ha<sup>-1</sup>. In nutshell, based on grain yield, net benefit, MRR, better quality traits, N rate of 92 kg ha<sup>-1</sup> could be recommended for both Lemu and Wane variety production around Mekdela area. However, as the experiment was conducted only for a single season, we suggest the repeat of the study for more seasons around Mekdela area and similar agroecology.

**Key words:** agronomic efficiency, bread wheat varieties, nitrogen fertilizer, nutrient uptake.

### INTRODUCTION

Wheat is the world's leading cereal grain and more than one-third of the population of the world uses it as a staple food (Curtis, 2002). It is one of the most important cereals cultivated in Ethiopia. It ranks third after sorghum (*Sorghum bicolor*) and maize (*Zea mays*) in area coverage, while second in total production after maize (CSA, 2015). Despite the large area coverage under wheat, the national average yield in Ethiopia is about 2.2 t ha<sup>-1</sup> which is below Kenya, Africa and the world's average of about 2.9, 2.4 and 3.2 t ha<sup>-1</sup>, respectively (CSA, 2013; FAO, 2014). This is attributed to

cultivation of unimproved and low yielding varieties, inadequate and erratic rainfall, poor agronomic practices, diseases and insect pests which are among the principal limitations to wheat production in Ethiopia (Gorfu and Hiskias, 2000).

Ethiopia is the second largest wheat producer in Sub-Saharan Africa next to South Africa (White et al., 2001). Wheat is one of the major staple crops in the country in terms of both production and consumption. In terms of caloric intake, it is the second most important food in the country behind maize (FAO, 2014). Wheat is mainly grown in the highlands of Ethiopia, which lies between 6 and 16° N and

35 and 42° E, at altitude ranging from 1500 to 2800 meters above sea level and with mean minimum temperatures of 6°C to 11°C (MOA, 2012). There are two types of wheat grown in Ethiopia: durum wheat, accounting for 60 percent of production, and bread wheat, accounting for the remaining 40 percent (Bergh et al., 2012). Oromia Regional State accounts for over half of national wheat production (54 percent), followed by Amhara (32 percent); Southern Nations, Nationalities and Peoples (9 percent); and Tigray (7 percent) (CSA, 2013). Nitrogen is subjected to more transformations than any other essential elements. The ultimate source of nitrogen is N<sub>2</sub> gas, which comprises approximately 78 percent of the earth's atmosphere. Inert N<sub>2</sub> gas, however; is unavailable to plants and must be transformed by biological or industrial processes into forms which are plant-available. As a result, modern agriculture is heavily dependent on commercial nitrogen fertilizer (USDA, 2011). Low soil fertility, especially nitrogen (N) deficiency, is one of the major constraints limiting wheat production in Ethiopian highlands (Teklu and Hailemariam, 2009). Thus, increased usage of N fertilizer is considered to be a primary means of increasing wheat grain yield and protein content in these areas. Wheat productivity of the country in terms of yield per unit area of land is very low due to poor agronomic and soil management practices. Furthermore, inadequate level of technology is also among the constraints to increased wheat production in the highlands and mid highlands of Ethiopia (Demeke and Marcantonio, 2013). As a result of this, the Ethiopian government is forced to import wheat every year because of higher demand than supply. To increase and sustain wheat production and to narrow down the gap between supply and demand, adoption of proper soil fertility managements is of paramount importance. For the improvement of soil fertility, uses of both chemical and organic fertilizer become very vital. Although there is potential for further crop yield improvement, the use of chemical fertilizers has made a significant contribution to crop yield increases so far (Asnakew et al., 1991). Nevertheless, fertilizers are applied by less than 45% of farmers, on about 40% of the cultivated land for crop production, most likely below

optimal dosage levels are applied (Dercon and Hill, 2009). Nitrogen (N) is often the most deficient of all the plant nutrients in Ethiopia. Wheat is very sensitive to insufficient nitrogen and very responsive to nitrogen fertilization. Variety based fertilizer recommendation is unusual in Ethiopia. Diammonium phosphate (DAP) and Urea are the only fertilizers applied to all soil types in the country for soil fertility management. The blanket recommendation rate for these fertilizers is 150 kg DAP ha<sup>-1</sup> and 100-150 kg Urea ha<sup>-1</sup> for wheat production. These rates vary as per the agro-ecological domains of the country, but farmers apply below the recommended rate. The increase of agricultural food production worldwide over the past four decades has been associated with a 7-fold increase in the use of N fertilizers (Hirel et al., 2007). Therefore, application of nitrogen fertilizer at the right rate and time is vital for the enhancement of soil fertility and crop productivity.

Cropping system, soil and water management, use of appropriate N fertilizer and application rate based on crop variety and soil type are among the main management options to increase N fertilizer use efficiency (USDA, 2011). In addition, use of slow N releasing fertilizers, nitrification inhibitor, N efficient species or genotypes, and disease, insects and weeds control are also important factors to be considered to improve N use efficiency (Fageria, 2009). Excess N application rate leads to acidity and susceptibility to other biotic and abiotic factors. Therefore, optimum nitrogen fertilizer application rate should be studied to determine its effect on yield, yield components and quality of bread wheat varieties based on agro-ecologies and fertility status of the soil. Therefore, the objectives of this study were to evaluate the effect of N fertilizer rates, varieties and their interaction on yield, yield components of bread wheat varieties and to identify economically optimum N fertilizer rates and appropriate bread wheat variety for the study area.

## **MATERIALS AND METHODS**

### **Description of the Study Areas**

The study was conducted in Northern high land Ethiopia, of Amhara Regional State, Mekdela

district. The area is located at 11 29' 59.99"N latitude and 38° 44' 59.99" E longitudes and an altitude of 2953 masl. The study was conducted in 2019 crop season Mekdela District. It is found in South Wollo zone of Amhara Regional State and 550 km away from Addis Ababa. The main agricultural activities are carried out mixed crop livestock production system is found in both high and low land areas of South Wollo zone. The highland mixed crop livestock production system is largely based on intensive cultivation of cereals, pulses, tubers, vegetables and some oil crops. According to Ethiopian agro-ecological classification the area is grouped under dega with the major soil type Vertisols and the most dominate land cover taken by cultivated land.

### **Experimental Design, Treatments and Procedures**

The experiment consisted of 10 treatments as factorial combination of two improved bread wheat varieties *Lemu* (ETBW 6861) and *Wane* (ETBW 6130), and five rates of nitrogen (0, 46, 92, 138 and 184 kg ha<sup>-1</sup>) arranged in a randomized complete block design (RCBD) with three replications. Replications were folded into two blocks of five plots each to reduce heterogeneity within replications. Following the history of preceding production season, farmlands that were covered with wheat or barley in the preceding year were selected for planting. The gross plot size of the experiment was 4 m long and 2.6 m wide with 20 cm row spacing. The net harvestable plot size of the experiment was 3.20 m long and 2 m wide with 16 central rows. The spacing between plots and replications were 1 and 1.5 m, respectively.

### **Data collected**

#### **Agronomic parameters**

Data on the phenological, growth yield and yield component parameters were taken from the 16 central rows of harvestable area of the experimental plot as follows:

**Days to 50% heading:** numbers of days from date of sowing to the stage where 50% of the spikes have fully emerged were recorded.

**Days to 90% physiological maturity:** number of days from date of sowing to the stage where 90% of the plants in the plot reached physiological maturity was recorded.

**Days to grain filling:** it was obtained by the number of days to maturity minus the number of days to heading.

**Number of effective tillers:** it was recorded from randomly selected 10 plants in each experimental plot at physiological maturity.

**Plant height (cm):** it was measured from the ground level to the tip of spike excluding the awn at physiological maturity.

**Spike length (cm):** it was the length of the spike from the node where the spike emerges to the tip of the spike, excluding the awn.

**Grains per spike:** it was taken from ten randomly selected spikes per net plot at harvest and averaged per plant basis.

**Above ground dry bio-mass yields (kg ha<sup>-1</sup>):** it was recorded from 16 central rows after sun drying to a constant weight for each plot.

**Grain yield (kg ha<sup>-1</sup>):** grain yield was recorded from 16 central rows and then adjusted to 12.5% moisture level for each plot.

**Thousand grain weight (g):** it was the weight of 1000 seeds determined by carefully counting the grains harvest of each experimental plot by seed counter and weighing them using sensitive balance.

**Straw yield (kg ha<sup>-1</sup>):** was measured by subtracting the grain yield from the total above ground biomass yield (after threshing).

**Harvest index (HI %):** it was the ratio of dried grain weight to the dried total above ground biomass weight per plot multiplied by 100.

### **Data Analysis**

The collected data were subjected to analysis of variance (ANOVA) using SAS software program (SAS 9.0, 2002). Significant difference among treatment means were assessed using the Duncan Multiple Range Test (DMRT) at 0.05 level of probability (Gomez and Gomez, 1984).

## **RESULTS AND DISCUSSIONS**

### **Effects of Nitrogen Rate and Variety on Crop Phenology**

#### **Days to 50% heading**

Days to 50% heading was highly significantly ( $p < 0.001$ ) affected by variety and N-fertilizer rate, but not for their interaction. The bread wheat variety *Wane* (64.2 days) was 5 days earlier than *Lemu* (69.3 days). This difference

could be attributed to the genetic makeup. In line with this, bread wheat varietal differences with respect to heading were reported by (Jemal et al., 2015).

Increasing N-fertilizer rates from 0 to 184 kg ha<sup>-1</sup> extended the number of days to 50% heading from 55 to 81 days (Table 1). The use of low N-fertilizer rate in wheat shortens the intervals between the growth phases by facilitating the physiological activities of crops due to the accessibility of inadequate resource. Short number of days to heading was recorded for plots which received zero rates of nitrogen while the longest days to heading was recorded for plots that received 184 kg N ha<sup>-1</sup>. However, the two varieties did show a consistent increasing trend with increasing N-fertilizer rates. Getachew (2004) and Mekonen (2005) reported that days to heading were significantly delayed when N fertilizer was applied at the highest rate for wheat and barley production compared to the lowest rate. This could be due to the fact that higher N rates enhance more vegetative growth and larger photosynthesis than reproductive parts. Rashid et al. (2007) also reported that NP application significantly affected days to heading of barley.

### Days to 90% maturity

The results showed that the main effects of variety and N-fertilizer rate were highly significant ( $p < 0.001$ ) on days to 90% physiological maturity. Conversely, the interaction effect of N-fertilizer rates and variety did not show any significant effect on days to 90% physiological maturity. Variety *Wane* took shorter time to mature compared to variety *Lemu* (Table 1). It is also important to note that the differences in maturity can be caused by the combined effect of genetic and environmental factors during the growth stage of the crops. Days to maturity consistently increased with increasing N rate.

The shortest and longest days to maturity were recorded from 0 and 184 kg ha<sup>-1</sup> N rates, respectively. This might be attributed to the role of N, which increases vegetative growth of crops whereby it delays maturity time. Similar result was reported by Woinshet (2007) in which N-fertilizer supply delayed maturity in malt barley. These results were in line with Bekalu and Mamo (2016) who reported that, N fertilizer rate significantly affected days to maturity on wheat.

Table 1. Main effect of variety and N- rate on days to heading, days to maturity, number of effective tillers per plant and plant height in 2019 cropping season at Mekdela

Variety	DH	DM	ETL	PH(cm)
<b>Lemu</b>	72.33a	146.00a	5.08a	98.60a
<b>Wane</b>	67.40b	134.70b	4.76b	96.70b
SE ±	0.19	0.23	0.04	0.20
LSD (%)	0.55	0.69	0.12	0.61
<b>N (Kg<sup>-1</sup>ha)</b>				
<b>0</b>	65.00e	132.80e	4.30d	95.00d
<b>46</b>	67.67d	136.70d	4.66c	96.80c
<b>92</b>	70.00c	140.20c	5.73a	98.20b
<b>138</b>	72.00b	144.00b	5.13b	99.00ab
<b>184</b>	74.67a	148.20a	4.76c	99.30a
SE ±	0.29	0.37	0.06	0.32
LSD (%)	0.88	1.10	0.18	0.96
CV (%)	1.03	0.65	3.11	1.14

DH = Days to heading, DM = Days to maturity, ETL = Number of effective tillers per plant, PH = Plant height. Means followed by the same letter(s) within a column are not significantly different from each other at 5% level of significance, ns: not significant.

### Days to grain filling period

Days to grain filling period were significantly ( $p < 0.001$ ) influenced by the main effects of N rate, variety and their interaction. The longest days to grain filling period were at higher N rates, 138 and 184 kg ha<sup>-1</sup> for variety *Lemu* than *Wane* (Table 2). Despite small variability, both varieties showed a consistent increasing

trend in days to grain filling with increasing nitrogen fertilizer rates. About seven more days were required for the 184 kg N ha<sup>-1</sup> treatment, which took 77 days to grain filling for *Lemu* variety; while five days difference was noted for *Wane* variety with similar treatment compared to the control (Table 2). The result was in line with that of Sofonyas (2016) who

stated that a steady increasing trend in grain filling period with rates of N in wheat was observed. Wakene et al. (2014) also reported that application of N significantly prolonged number of days to heading and maturity and grain filling period in barley.

## Effects of Nitrogen Rate and Variety on Growth Parameters

### Plant height

Plant height was highly ( $p < 0.001$ ) affected by N-rate and variety, but their interaction was non-significant. Variety *Lemu* (98.6 cm) had higher plant height than *Wane* (96.7 cm). This variation could be related to the inherent character of the variety. Though inconsistent, their heights increased with increasing N rate. The highest plant height (99.3 cm) was recorded from the treatment with the highest N rate of 184 kg ha<sup>-1</sup>, while the shortest plant height was recorded on plots with 0 kg N ha<sup>-1</sup>. Tayebbeh et al. (2011) and Sofonyas (2016) reported significant increments in plant height due to application of high nitrogen rate.

### Spike length

Spike length was significantly influenced by the main effects of N rate, and variety. It was also highly significantly ( $p < 0.001$ ) affected by the interactions of both N rate and variety. Results of N rates and variety interaction effects indicated that the longest spike length (9.33 cm) was obtained from 92 kg N ha<sup>-1</sup> for *Lemu* whereas 138 kg N ha<sup>-1</sup> resulted in the longest spike (7.13 cm) for *Wane*. The spike length of *Lemu* increased with increasing N supply from 0 to 92 kg ha<sup>-1</sup> and then decreased

from 138 to 184 kg N ha<sup>-1</sup> while for *Wane* variety it increased from 0 to 138 kg ha<sup>-1</sup> N rates. But no statistical difference was observed between 92 and 138 kg ha<sup>-1</sup> N rates for *Lemu* variety (Table 2). These findings justified that N fertilizer application by itself does not have significant effect on spike length of wheat. In line with these results, Bekalu and Mamo (2016) reported that optimum amount of fertilizer application has significant effect on spike length growth. Similarly, Smith and Hamel (1999) reported that excessive application of N fertilizer has toxic effect on wheat growth and results in stunted growth and reduced spike length.

### Number of effective tillers

Number of effective tillers were highly ( $p < 0.001$ ) significantly influenced by N-rate and variety, but their interaction was non-significant. Corresponding to this growth parameter, variety *Lemu* had better performance than variety *Wane* (Tables 1). The response of the crop in terms of number of effective tillers was higher at 92 kg N ha<sup>-1</sup>, but the trend was inconsistent for the remaining N rates. The lowest numbers of effective tillers were recorded from the control plots; which might be due to the role of N in accelerating vegetative growth of plants. The results were in agreement with that of Abdullatif et al. (2010) who reported that increasing in the number of effective tillers with nitrogen fertilization. Bereket et al. (2014) and Abdollahi et al. (2012) also reported that nitrogen fertilization have significant effect on effective number of tillers of wheat.

Table 2. The effects of N rates and bread wheat variety interactions on days to grain filling and spike length in 2019 cropping season at Mekdela

	Days to grain filling		Spike Length(Cm)	
	Variety		Variety	
N (Kg <sup>-1</sup> ha)	Lemu	Wane	Lemu	Wane
0	70.67cd	65.00g	6.27e	6.01e
46	71.67bc	66.33fg	7.13c	6.46de
92	72.67b	67.67f	9.33a	6.93cd
138	76.00a	68.00ef	9.27a	7.13c
184	77.33a	69.67de	8.47b	6.87cd
SE ±	0.56		0.16	
LSD (%)	1.67		0.47	
CV (%)	1.38		3.75	

Means followed by the same letter(s) within columns of days to grain filling and spike length are not significantly different from each other at 5% level of significance.

## Effects of Nitrogen Rate and Variety on Yield and Yield Components

### Number of seeds per spike

The analysis of variance showed significant difference ( $p < 0.01$ ) on number of seeds per spike due to the main effects of variety and rates of nitrogen, but their interactions were not significant. Higher number of seeds per spike (54.6) was recorded in variety *Lemu* than *Wane* (53.4) (Table 3). Lower number of seeds per

spike was recorded in control plot than fertilized plots. Number of seed per spike increased from 0 to 92 kg N ha<sup>-1</sup> and then decreased from 138 to 184 kg N ha<sup>-1</sup>. Despite this difference, the highest seed per spike (57.4) obtained from the plots fertilized with 92 kg ha<sup>-1</sup> N rate was statistically at par with that of 138 kg ha<sup>-1</sup> N rate (Table 3). The results were in conformity with that of Tayebbeh et al. (2010) who stated that increasing N rates up to optimum level significantly increased number of seed spike<sup>-1</sup>.

Table 3. Main effect of variety and N- rate on SPS, TSW (g), GY (kg ha<sup>-1</sup>), SY (kg ha<sup>-1</sup>) HI (%) and NHI (%) at Mekdela in 2019 main cropping season

Variety	SPS	TSW(g)	SY	HI (%)	NHI (%)
<b>Lemu</b>	54.6a	38.3a	13274.2a	32.5	65.7
<b>Wane</b>	53.4b	42.4b	12575.5b	33.0	63.5
SE ±	0.3	0.6	171	0.25	1.3
LSD (5%)	0.81	1.69	506.9	NS	NS
<b>N (Kg<sup>-1</sup>ha)</b>					
0	48.5d	37.4c	11648.0d	26.5d	61.6b
46	52.6c	39.5bc	12305.0cd	34.3b	68.7a
92	57.4a	44.5a	12948.0bc	36.3a	70.0a
138	56.5a	40.4b	13350.0b	34.5b	62.1b
184	39.9bc	14373.0a	14373.0a	32.0c	60.0b
SE ±	0.9	0.9	269.7	7.04	2.1
LSD (5%)	1.3	2.68	801.4	1.2	6.2
CV (%)	1.9	5.5	5.1	2.9	7.9

SPS = No. of seed spike-1, TSW = Thousand seed weight, SY = Straw yield and HI = Grain harvest index, NHI = Nitrogen harvest index. Means followed by the same letter(s) within column are not significantly different from each other at 5% level of significance, ns: not significant.

### Thousand Seed weight

The results revealed that the main effect of varieties and N rates showed highly significant ( $p < 0.001$ ) difference with respect to thousand seed weight, but not for their interaction (Table 3). Variety *Wane* exceeded variety *Lemu* by 10.7 % in thousand seed weight. This could be due to the late maturity of variety *Lemu* which might have suffered from unfavorable environmental condition lately in the growing season.

The highest thousand seed weight (44.5 g) was produced by the application of 92 kg N ha<sup>-1</sup>, while there was inconsistent trend of thousand seed weight in the remaining N rates. That is why; the result was in contrary to Tayebbeh et al. (2011) who reported number seeds spike<sup>-1</sup> and 1000 grain weight were significantly enhanced by increasing nitrogen levels. However, the results were aligned with Abdollahi et al. (2012) and Khan et al. (2013) whereby nitrogen rates and sources significantly influence thousand seed weight.

### Grain yield

The results revealed that main effect of varieties and N rates showed highly significant ( $p < 0.001$ ) difference with respect to grain yield, but their interaction was not significant. *Lemu* variety was more productive than *Wane*. The relative advantage in grain yield of *Lemu* (6445 kg ha<sup>-1</sup>, +2.3%) of this variety might be largely attributed to its higher number of seeds per spike, number of effective tillers and spike length. This result is in agreement with Solomon et al. (2000) who reported a notably superior performance of between bread wheat varieties. Irrespective of N rate, more grain yield (7415 kg ha<sup>-1</sup>) was obtained by the application of 92 kg N ha<sup>-1</sup>. Nitrogen fertilizer applied at rate of 138 kg ha<sup>-1</sup> had 6.2 % less and 66.4 % more grain yield than fertilizer rate applied at 92 kg ha<sup>-1</sup> and control, respectively. In line with the result of this study, Bereket et al. (2014) reported that increasing rate of nitrogen fertilization increased grain yield of wheat. There were significant decreases in

grain yield when the rate of N increased from 92 to 184 kg ha<sup>-1</sup>. This is because, balanced supply of N at optimal amount results in higher net assimilation rate and increased grain yield. The present study showed, maximum grain yield of wheat could be obtained by applying of 92 kg ha<sup>-1</sup> and beyond this rate; applied N becomes in excess of the crop need and consequently the yield decreased.

### Straw yield

The main effect of N fertilizer rate and variety exhibited highly ( $p < 0.001$ ) differential responses in straw yield, but their interaction was not significant. *Wane* variety was less productive than *Lemu* variety. *Lemu* variety gave 699 kg ha<sup>-1</sup> more straw yield advantage than *Wane* variety (Table 3). This difference might be attributed to the higher productivity of yield and yield components of *Lemu* variety. Straw yield increased with increasing N rates, whereby the lowest and highest straw yields were obtained from control plots (11648 kg ha<sup>-1</sup>) and from plots that received 184 kg N ha<sup>-1</sup> (14373 kg ha<sup>-1</sup>), respectively (Table 3). Nitrogen increases vegetative growth of plants, especially at higher doses. Besides, the significant increase in plant height, spike length and number of fertile tillers by N rate contributed to the significant increase in straw yield. In agreement with this result, Abebe (2012) and Bereket et al. (2014) reported that wheat straw yield increased with N rates.

### Harvest index and Nitrogen harvest index

Harvest Index (HI) and nitrogen harvest index (NHI) were highly ( $p < 0.01$ ) significantly influenced by N rate, but the main effect of varieties and the interaction between the two factors were non-significant. Even if the two varieties were not significantly different from each other, variety *Wane* gave 0.5% more HI than variety *Lemu* whereas variety *Lemu* 2.2% more NHI than variety *Wane* (Table 3). This could be due to increasing in biological yield which is inversely proportional to HI and the lower total N uptake in *Wane* variety which is inversely proportional to NHI. Significant varietal differences on harvest index in bread wheat varieties were also reported by Jemal et al. (2015) and cultivars differ for nitrogen harvest index (Metho et al., 1997). With increasing N rates from 0 to 92 kg N ha<sup>-1</sup>, there

were increasing trends of yield and yield components. However, HI and NHI significantly decreased with increasing N rates above 92 kg N ha<sup>-1</sup> (Table 3). It might be due to nitrogen harvest index and grain harvest index were used to indicate the physiological adaptations of crops to soil N availability, as they expressed by the allocation of N and biomass, respectively, to grain in relation to the whole aboveground plant. A mean harvest index of about 50% with a positive trend due to increasing N rate was previously reported in Ethiopia (Taye et al., 2002). In contrast, Marcelo et al. (2013) reported that rates and sources of N did not affect harvest index of wheat.

### CONCLUSIONS

Ethiopia is the second largest wheat producer in Sub-Saharan Africa next to South Africa. Wheat productivity in the country in terms of yield per unit area of land is very low due to poor agronomic and soil management practices. Low soil fertility is particularly one of the factors limiting crops productivity in the highlands of Ethiopia. To this effect, the N fertilizer rates and its interaction, on nitrogen use efficiency, yield, yield components of newly released improved bread wheat varieties was conducted at Mekdela District during the 2019 cropping season. Two improved bread wheat varieties namely *Lemu* (ETBW 6861) and *Wane* (ETBW 6130) were planted using five levels of nitrogen (0, 46, 92, 138 and 184 kg ha<sup>-1</sup>) in randomized complete block design with factorial arrangement in three replications. Urea was used as source of N in two split forms, at planting and at flowering times. Days to 50% heading, days to 90% physiological maturity, plant height, number of effective tillers, number of seeds per spike, thousand seed weight, grain yield, and straw yield were significantly affected by main effects of variety and N fertilizer rate, but not by their interactions. Grain harvest index and nitrogen harvest index were highly and significantly affected by N fertilizer, but the main effect of varieties and the interaction between N and variety were non-significant. Corresponding to growth parameters, variety *Lemu* had better performance than variety *Wane*. *Lemu* (6445 kg

ha<sup>-1</sup>) variety was more productive than the early maturing *Wane* (6259 kg ha<sup>-1</sup>) variety at N rate of 92 kg N ha<sup>-1</sup>. With respect to quality, *Lemu* variety had the highest score for hectoliter weight (79.99) than the variety *Wane* (79.43), whereas variety *Wane* had 6.57 % wet gluten and 2.24% dry gluten higher than variety *Lemu*. Grain N uptake was significantly different for varieties, but not for N rate and their interaction. The main effect of N rates showed significant (p<0.05) difference for agronomic efficiency. No significant difference was observed in agronomic, recovery and physiological efficiencies between varieties and their interaction. Agronomic, recovery and physiological efficiencies decreased with increasing N rates. The highest values were recorded at 46 kg N ha<sup>-1</sup> for three agronomic, recovery and physiological efficiencies. Analytical results of the soil sample after harvesting revealed that total N was higher and the highest value was obtained from application of 92 kg N ha<sup>-1</sup>. Partial budget analysis indicated that nitrogen rate of 92 kg N ha<sup>-1</sup> economically beneficial with MRR of 980% compared to the other treatments in this study. Generally, based on grain yield, net benefit, MRR, better quality traits, N rate of 92 kg ha<sup>-1</sup> could be recommended for both *Lemu* and *Wane* variety production around Mekdela area.

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## COMPARATIVE EVALUATION OF INCREASED RATES OF SEKATOR OD ON THE EFFICACY AND GROWTH TRAITS IN WINTER WHEAT

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### Abstract

*In the winter wheat vegetation period of 2019/2020 a field experiment with Sekator OD (100 g/l amidosulphuron + 25 g/l iodosulfuron + 250 g/l mefenpyr-diethyl) was conducted. The trial was situated on the experimental field of the Agricultural University of Plovdiv, Bulgaria. Variants of the trial were: 1. Untreated control; 2. Sekator OD - 0.10 l ha<sup>-1</sup>; 3. Sekator OD - 0.15 l ha<sup>-1</sup> and 4. Sekator OD - 0.20 l ha<sup>-1</sup>. The herbicide application was performed in tillering stage (BBCH 21-29) in spring. For the study's purposes, the "Avenue" variety was grown. As well as the efficacy and the selectivity of the herbicide product, several quantitative and qualitative indicators were evaluated. The highest efficacy against the existing broadleaf weeds for the rate of 0.20 l ha<sup>-1</sup> was recorded. There were no visual phytotoxic symptoms for the crop for any of the evaluated rates. By using the statistical criterion of Student Test, a positive and significant increase in the values of stem height at the rate of 0.10 l ha<sup>-1</sup> and the number of ears at the rate of 0.20 l ha<sup>-1</sup> were found.*

**Key words:** *Triticum aestivum L., efficacy, biometry, herbicide, yield.*

### INTRODUCTION

The high weed infestation in winter wheat (*Triticum aestivum* L.) can decrease the yields up to 70% (Tonev et al., 2011; Tonev et al., 2007; Bekelle, 2004). The effective weed management in winter field crops is a main part of successful agricultural production (Brooke and McMaster, 2016). Weed control in winter wheat is performed by applications of different protective and agricultural measures, as well as herbicide applications in dependence of the dominating weeds (Markovic et al., 2005).

Depending on the crop management and the meteorological conditions, the different weeds can be in different density and can form different weed associations (Dimitrov et al., 2016).

The choices of relevant herbicide product, optimal application period, as well as phenological stage of treatment are one of the most important points of the crop management (Abbas et al., 2009; Khalil et al., 2008; Sherawat and Ahmat, 2005). In the modern agriculture the weed control is mainly performed by the chemical method. A number of authors study the selectivity and efficacy of different herbicides in crops (Marinov-Serafimov and Golubina, 2015; Rankova and

Tityanov, 2015; Hristova, 2007; Atanasova, 2002). Most of the herbicide products control only a specific group of weeds, and for assuring wide spectrum or weed control it is recommended to use herbicide combinations (Chaudhry et al., 2008; Bostrom and Fogelfors, 2002).

The aim of our study is to evaluate the efficacy of increasing rates of the herbicide product Sekator OD and its influence on some growth parameters of winter wheat.

### MATERIALS AND METHODS

The experiment was situated in the experimental field of the Base for Training and Implementation of the Agricultural University of Plovdiv, Bulgaria. The trial was conducted by the randomized block design in 3 replications. The size of the experimental plot was 10 m<sup>2</sup>. The trial included the following treatments: 1. Untreated control; 2. Sekator OD - 1.0 l ha<sup>-1</sup>; 3. Sekator OD - 1.5 l ha<sup>-1</sup> and 4. Sekator OD - 2.0 l ha<sup>-1</sup>.

For evaluating of the efficacy, the 10-score scale of EWRS (European Weed research Society) was used. The herbicide application was accomplished in BBCH 21-29 (tillering stage in spring). In the study, the "Avenue" variety was grown.

On the whole experimental field combined fertilization with 250 kg ha<sup>-1</sup> with N:P:K (15:15:15), followed by deep ploughing on 25 cm of depth was done. Before sowing of the crop, disk harrowing, two harrowings, as well as spring dressing with 250 kg ha<sup>-1</sup> NH<sub>4</sub>NO<sub>3</sub> was also applied.

The processing of the initial data was done with the statistical package of SPSS 19 program and the comparisons were made once with the control treatment and the second time with each variant with each in order to determine more precisely the influence of the rate of the herbicide product on the change of the values of the studied traits of winter wheat.

With the help of the Student's Test criteria (t) the levels of reliability of the differences between the treatments at calculated probability 5%, 1% and 0.1%. The groups to which the individual variants are included are defined, and the control is always in group IV.

The parameters evaluated: stem height (cm), ear length (cm), ear number, number of grains per ear, grain weight per ear, absolute seed mass, as well as grain yield. The data for the grain yield were processed by Duncan's multiple range test ( $p < 0.05$ ).

## RESULTS AND DISCUSSIONS

The natural weed infestation on the experimental field was presented by *Sinapis arvensis* L., *Anthemis arvensis* L., *Papaver rhoeas* L. and *Fumaria officinalis* L.

On Table 1 is the obtained data for the efficacy of the studied herbicide product against *S. arvensis*.

Table 1. Efficacy of Sekator OD against *S. arvensis*, %

Treatments/days after application	14 <sup>th</sup>	28 <sup>th</sup>	56 <sup>th</sup>
1. Untreated control	-	-	-
2. Sekator OD - 0.10 l ha <sup>-1</sup>	85	95	100
3. Sekator OD - 0.15 l ha <sup>-1</sup>	90	95	100
4. Sekator OD - 0.20 l ha <sup>-1</sup>	95	100	100

The efficacy of the Sekator OD is excellent for all evaluated rates of the herbicide product. On the last reporting date it is 100% independently the application rate of Sekator OD. These findings correspond with the results of previous our study where 100% efficacy against this

weed on the 56<sup>th</sup> day after treatment was recorded (Mitkov et al., 2017)

The efficacy of Sekator OD against *A. arvensis* is presented on Table 2. The efficacy of the herbicide was also excellent for all evaluated rates against this weed. The efficacy was lower 14 days against the weed and varied between 60-70%. On the last reporting date the efficacy was 100% for the rates of Sekator OD 0.15 and 0.20 l ha<sup>-1</sup>. The dose of 0.10 l ha<sup>-1</sup> was 90%. Several researchers have found that *A. arvensis* can be controlled by amidosulfuron + iodosulfuron (Sekator 6.25 WG; Sekator and Sekator Progress) (Vilau et al., 2010; Soroka and Soroka, 2003; Adamczewski and Miklaszewska, 2001).

Table 2. Efficacy of Sekator OD against *A. arvensis*, %

Treatments/days after application	14 <sup>th</sup>	28 <sup>th</sup>	56 <sup>th</sup>
1. Untreated control	-	-	-
2. Sekator OD - 0.10 l ha <sup>-1</sup>	60	80	90
3. Sekator OD - 0.15 l ha <sup>-1</sup>	65	85	100
4. Sekator OD - 0.20 l ha <sup>-1</sup>	70	85	100

The efficacy of Sekator OD against *P. rhoeas* is on Table 3. It was found that the lowest rate of 0.10 l ha<sup>-1</sup> cannot assure sufficient control against this widely spread weed species. On the 14<sup>th</sup> day after treatment, the efficacy of Sekator OD - 0.10 l ha<sup>-1</sup> was 35% only. It increased up to 50% on the second evaluation date and reached 65% only on the 56<sup>th</sup> day after application.

Table 3. Efficacy of Sekator OD against *P. rhoeas*, %

Treatments/days after application	14 <sup>th</sup>	28 <sup>th</sup>	56 <sup>th</sup>
1. Untreated control	-	-	-
2. Sekator OD - 0.10 l ha <sup>-1</sup>	35	50	65
3. Sekator OD - 0.15 l ha <sup>-1</sup>	75	85	95
4. Sekator OD - 0.20 l ha <sup>-1</sup>	85	95	100

In our previous study, the efficacy of the examined rate of Sekator OD (0.12 l ha<sup>-1</sup>) did not fully control *P. rhoeas*. The obtained efficacy on the 56<sup>th</sup> day after treatments was 80% (Mitkov et al., 2018). In the present trial, the rates of 0.15 and 0.20 l ha<sup>-1</sup> for Sekator OD assured excellent efficacy.

The efficacy of Sekator OD against *F. officinalis* is presented on Table 4. According

to Montazeri et al. (2005) *F. officinalis* was relatively tolerant to all evaluated herbicides.

Table 4. Efficacy of Sekator OD against *F. officinalis*, %

Treatments / days after application	14 <sup>th</sup>	28 <sup>th</sup>	56 <sup>th</sup>
1. Untreated control	-	-	-
2. Sekator OD - 0.10 l ha <sup>-1</sup>	20	25	25
3. Sekator OD - 0.15 l ha <sup>-1</sup>	25	35	40
4. Sekator OD - 0.20 l ha <sup>-1</sup>	35	40	45

In our study the efficacy results were not sufficient for any of the evaluated herbicide

rates. The efficacy against *F. officinalis* varied from 25 to 45% with increasing of the Sekator OD's doses.

The data for the growth parameters and its statistical analyses is on Table 5.

The meanings of t are as follows: 2.00 (p<0.05); 2.66 (p<0.01); 3.46 (p<0.001).

The indicator stem height is with significantly higher values at the treatment with Sekator OD - 0.10 l ha<sup>-1</sup> in comparison to the other treatments at p<0,001.

Table 5. Data analyses for 5 quantitative parameters of wheat (growth and productive)

Stem height (cm)					
Treatments	Average	Difference	t e	Significance	Group
1	85.00	-	-		IV
2	90.33	5.33	3.94	+++	I
3	84.90	-0.10	0.02	ns	IV
4	84.70	-0.30	0.22	ns	IV
Tr. 2 and 3		5.43	4.56	+++	I
Tr. 2 and 4		5.63	5.02	+++	I
Tr. 3 and 4		0.20	0.22	ns	IV
Ear length (cm)					
Treatments	Average	Difference	t e	Significance	Group
1	9.43	-	-		IV
2	9.14	-0.29	1.88	ns	IV
3	9.15	-0.28	1.38	ns	IV
4	9.66	0.23	1.31	ns	IV
Tr. 2 and 3		-0.01	0.01	ns	IV
Tr. 2 and 4		-0.52	3.56	+++	I
Tr. 3 and 4		-0.51	2.79	++	II
Ear number					
Treatments	Average	Difference	t e	Significance	Group
1	21.43	-	-		IV
2	21.16	-0.27	0.95	ns	IV
3	21.17	-0.26	0.74	ns	IV
4	22.20	0.77	2.37	ns	IV
Tr. 2 and 3		0.01	0.04	ns	IV
Tr. 2 and 4		1.04	3.85	+++	I
Tr. 3 and 4		1.03	3.22	++	II
Number of grains per ear					
Treatments	Average	Difference	t e	Significance	Group
1	41.20	-	-		IV
2	47.50	6.30	2.60	+	III
3	53.30	12.1	4.89	+++	I
4	54.85	13.6	5.51	+++	I
Tr. 2 and 3		5.80	2.15	+	III
Tr. 2 and 4		7.35	2.72	++	II
Tr. 3 and 4		1.55	0.57	ns	IV
Grain weight per ear					
Treatments	Average	Difference	t e	Significance	Group
1	122	-	-		IV
2	1.75	0.53	3.94	+++	I
3	2.005	0.78	6.28	+++	I
4	2.05	0.83	7.16	+++	I
Tr. 2 and 3		0.25	2.02	+	III
Tr. 2 and 4		0.30	2.61	+	III
Tr. 3 and 4		0.045	0.48	ns	IV

For two of the studied parameters (ear length and ear number), an overall tendency was found - for all treated variants, non-significant differences with the untreated control was recorded (ns). The effect of the herbicide application and its rate was revealed for treatments 2 and 4 - for the parameter ear length) and for treatment 4 - for the ear number when the comparisons are performed between treated variants (for significance 1% and 0.1%). The higher rates of Sekator OD that showed higher weed control allow winter wheat to develop significantly higher ear number, as well as longer ears.

The results obtained clearly showed the positive effect of the treatment with Sekator OD for the two productive indicators - number of grains per ear and grain weight per ear. At treatments 3 and 4 the significant difference when compared with the untreated control at  $p < 0,001$  determines the rates of 0.15 и 0.20  $l\ ha^{-1}$  in the highest group (I).

For the parameter grain weight per ear it was found that the three evaluated rates of Sekator OD showed equal effect when compared to the untreated control at level of significance  $p < 0,001$ .

In the comparison between the studied variants, for the treatments with the rates of Sekator OD of 0.15 and 0.20  $l\ ha^{-1}$ , a tendency of significant differences according to treatment 2 (Sekator OD - 0.20  $l\ ha^{-1}$ ) at  $p < 0.01$  and  $p < 0.05$  was recorded. These findings confirm that application of Sekator OD in higher rates, which removes the concurrence of the crop with the weeds, allows the winter wheat plants to show its productive abilities.

On Table 6 is the obtained data for the absolute seed mass.

Table 6. Percent relation of the absolute seed mass (g)

Treatments/days after application	Mass (g)	%	Sign.
1. Untreated control	29.66	100.0	
2. Sekator OD - 0.10 $l\ ha^{-1}$	36.97	124.0	+++
3. Sekator OD - 0.15 $l\ ha^{-1}$	37.61	126.8	+++
4. Sekator OD - 0.20 $l\ ha^{-1}$	37.45	126.2	+++

The absolute seed mass is very important quality indicator (Mehmood et al., 2014). On one hand, for the treated with Sekator OD variants the difference varied with very low values - from 0.6% to 2.8%. The results

recorded proved that with increasing the rate of the studied herbicide product, the absolute seed mass is not significantly influenced. On the other hand, the comparison of the untreated control with all treated variants showed pronounced differences at level of significance  $p < 0,001$ .

The intensive weed infestation can rapidly decrease the grain yield at winter wheat (Mitkov et al., 2018; Mitkov et al., 2017; Walia et al., 2011; Delibaltova et al., 2009; Walia and Singh, 2007; Bekelle, 2004). The choice of proper herbicide, optimal time and rate of application are one of the most important moments for growing winter wheat (Sherawatand, 2005).

The results regarding the yields obtained in our study are on Figure 1.

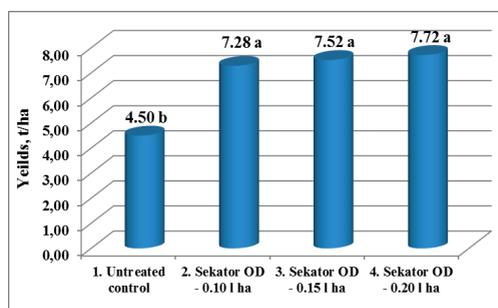


Figure 1. Winter wheat grain yields,  $t\ ha^{-1}$

Values with different letters are with proved difference according to Duncan's multiple range test ( $p < 0.05$ )

All studied rates of Sekator OD contributed for obtaining higher yields in comparison with the productivity of the untreated control. The data for the yields of all treated variants are proved differences with the productivity of the untreated control according to Duncan's multiple range test ( $p < 0.05$ ).

## CONCLUSIONS

The weeds *Sinapis arvensis* L. and *Anthemis arvensis* L. can be successfully controlled by the lowest examined rate of Sekator OD (0.10  $l\ ha^{-1}$ ). The weed *Papaver rhoeas* L. can be controlled by the increased rates of Sekator OD (0.15  $l\ ha^{-1}$  and 0.20  $l\ ha^{-1}$ ).

The weed species *Fumaria officinalis* L. cannot be successfully controlled by any of the evaluated rates of Sekator OD.

Visual phytotoxic symptoms for the crop from any of the evaluated Sekator OD doses were not observed.

From the conducted experiment with the winter wheat variety “Avenue” under the conditions of high weed infestation with *Sinapis arvensis* L., *Anthemis arvensis* L., *Papaver rhoeas* L. and *Fumaria officinalis* L. we can summarize that the most significantly influenced productive parameters by the herbicide application are the number of grains per ear and the grain weight per ear, as well as the absolute seed mass, which are one of the most important yield-forming indicators.

The stem height is significantly influenced from the Sekator OD’s rate of 0.10 l ha<sup>-1</sup>, while the number of ears - from the rate of 0.20 l ha<sup>-1</sup>. All treated with Sekator OD variants had higher grain yields in comparison to the untreated control.

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## STUDY THE PRODUCTIVITY OF COMMON WHEAT VARIETIES

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### Abstract

*The main goal in the present study is to study the adaptability and productivity of common wheat varieties. The study was conducted in 2017-2019, in the Department of Plant Breeding at the Faculty of Agriculture at the Trakia University, Stara Zagora. The object of study are the productivity and structural elements of yield in several varieties of common wheat - Ingenio, Dalara, Moison, Falado, Gabrio, Pibrak (from the variety list of Syngenta) and Factor (Bulgarian selection). According to the two-factor analysis of variance, both the influence of the two factors (conditions of the year and variety) separately and their interaction, statistically proven with a very high degree of reliability ( $p \leq 0.001$ ) is the impact on the indicator - yield. The strongest influence on the variation of the trait is exerted by the factor year (37%), followed by the interaction between them (35%) and the factor variety (28%). In terms of the yield of wet gluten with the highest content, Dalara (30.8) stands out, on average for the period. Climate conditions have a great influence on DMG and it is expressed in the fluctuations of its values in Ingenio (from 22.9 to 32.4), in Gabrio (from 31.6 to 21.6) and in Factor (25.6- 29.0). Despite the changing environmental conditions, a small range of DMG variation was reported in Dalara (30.5 -31.0), in Moysen (27.1-28.0), in Falado (30.1-30.0), Pibrak (28.2-29.3).*

**Key words:** variety, common wheat, yield, crude protein, yield wet gluten.

### INTRODUCTION

The importance of wheat in the past is great and continues to be important in human nutrition today. In today's market conditions, increasing the yield and improving the quality of common wheat grain is becoming increasingly important.

Factors that affect productivity, such as climate change, dwindling natural resources, and others, lead to a reduction in crop productivity potential (Easterling, 2007; Lobell et al., 2008; Batiste et al., 2009; Tsenov et al., 2009; Nelson et al., 2010; Sevov et al., 2013).

Increasing the average temperature leads to variability in the productivity of all crops, prove Tigchelaar et al. (2018) in their research. For each degree of increase in average global temperature, yields are expected to decrease by an average of 7.4% for corn, 6.0% for wheat, 3.2% for rice and 3.1% for soybeans (Zhao et al., 2017).

The potential of common wheat is mostly measured by its productivity. The biological potential of each crop is not only genetically

determined, but is also influenced by growing conditions (Bazitov et al., 2010; Hristov, 2013; Delchev, 2012; Kuneva et al., 2014). Results obtained under different soil and climatic conditions show the ecological plasticity and adaptability of common wheat varieties (Penchev et al., 2004).

The climatic features of each region are leading in the selection of cultivated varieties. During the individual years of the study, conducted with 20 varieties of soft wheat Samodova (2008) proved the influence of climate change and the coincidence of the phases of plant development with the current climate conditions. The correct varietal structure depending on the specific agro-ecological conditions of the region can significantly increase the yields and the quality of the production (Ilieva, 2011).

The data analysis consisted of 13 lines grown in France in 14 environments (combinations of two years, four locations and two treatments). The grain yield and the date of laying are measured, and the environment is characterized by climatic data (water deficit, radiation,

temperature above 25°C) and others. The influence of the environment is also assessed by the stability of yields by other researchers Gordana et al. (2014) and Döringa et al. (2015). The main goal in the present study is to study the adaptability and productivity of common wheat varieties.

## MATERIALS AND METHODS

The study was conducted in 2017-2019, in the Department of Plant Breeding at the Faculty of Agriculture at the Trakia University, Stara Zagora. The experimental field is located in the region of Eastern Central Bulgaria, covering the Thracian lowland, located at 42° 41'51.75 " north latitude, 23°19'18.722 " east longitude and 169 m above sea level. According to the climatic zoning, the region falls within the European-continental area and the Transitional-Continental sub-area from it. The experiment was conducted in the experimental field, with soil type typically meadow-cinnamon soil. The thickness of the humus horizon in this soil species varies widely from 0.30 m to 0.75 m. According to its mechanical composition, the soil type is sandy-clayey.

The study was based on the method of fractional plots. The object of study are the productivity and structural elements of yield in several varieties of common wheat - Ingenio, Dalara, Moison, Falado, Gabrio, Pibrak (from the variety list of Syngenta) and Factor (Bulgarian selection). The study was performed by the method of fractional plots, in four replications. The size of the experimental plot is 10 m<sup>2</sup>.

In terms of humus and nutrient content, the soil is characterized as suitable for growing wheat. The soil is on average stocked with humus - 3.93%, on average stocked with mineral nitrogen - 40.8 mg/1000 g of soil. During the vegetation, a single feeding with ammonium nitrate was carried out. The amount of applied nitrogen fertilizer applied per hectare is in dose N<sub>140</sub>.

Phenological and biometric observations were performed. A qualitative analysis of the grain of the studied varieties was performed.

The obtained experimental data were statistically processed by computer software MS Excel. The assessment of the strength of

the influence of factors was calculated by the method of Plohiniski (Lakin, 1990). It is defined as part of the intergroup variation in the total variation. Work with the sum of the squares and calculate by the formula:

$$h_x^2 = \frac{D_x}{D_y}$$

Where:  $D_x$  - sum of the squares of the factor x,  $D_y$  - total sum of the squares (SS). The influence of both the irrigation factor and the year and their interaction has been established.

## RESULTS AND DISCUSSIONS

The development of common wheat takes place in different weather conditions. Figure 1 shows the dynamics of the average daily air temperatures for the study period. The same figure shows the uneven amount of rainfall during the growing season of the crop.

The analysis of the data shows the trends in the change of temperatures during the vegetation period of the crop. The study period is characterized by high stress of meteorological factors. Temperatures are close to the norm for the period 1930-2019. The first year is characterized by negative temperatures in January. There is a tendency to increase the average daily temperatures in the last two years. The total temperature in the second experimental year was 8.8% higher than the norm for a multi-year period (1930-2019).

The average annual rainfall for the period 1930-2019 is 436.1 mm. In the first economic year the amount of precipitation was 14.2% less than the norm for the long-term period. Rainfall of 0.9 mm was registered in December. The second year is characterized by a higher amount of precipitation. Precipitation of 621.9 mm was measured, which is 42.6% higher than the norm. Increased humidity and high temperatures are unfavorable processes for the grain, which reduce the starch content and hectolitre number.

Figure 2 shows the yields of common wheat varieties for the study period. The analysis of the results shows that the varieties Falado and Gabrio are superior to the other varieties in terms of grain yield.

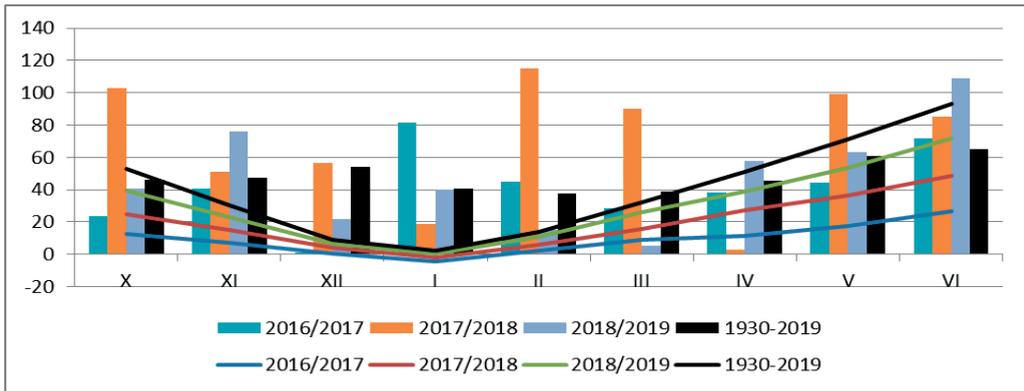


Figure 1. Climatogram for the period of development of common wheat, for the region of Stara Zagora, 2016-2019

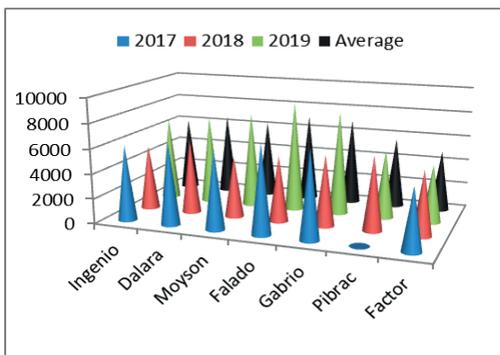


Figure 2. Productivity of common wheat varieties, kg/ha

The data show an excess of 44.7% for the Falado variety and 42.9% for the Gabrio variety. The results register good adaptability and productivity in specific climatic conditions. Ingenio has 22.6% higher yields than the standard.

For the Moyson variety the excess is 28.8%, and for the Dalara variety it is 33.8%. In the case of the Pibrac variety, the excess of almost 16.0% was reported in two years. The variety is new and was registered only in 2018. The performed analysis of variance for the influence of factors year, variety and their interaction on the indicator "yield" is reflected in Table 2. For the indicator "yield" the strongest influence of environmental factors (year) is observed with a dominant influence of 37% and with clear reliability  $p \leq 0.001$  on the change of the indicator. In second place is the interaction of the two factors respectively by 35%, followed by the variety factor with an impact of 28%.

Wheat grain has a different composition depending on the type, variety and growing conditions. Of the grain composition, protein substances are of the greatest importance. Climate plays a big role in the content of protein in the grain. The drier and more continental it is, the richer the grains of cereals are in protein.

The protein substances in the grain of individual plants have different properties and therefore not every grain can produce good quality bread. Wheat proteins are found mainly in the form of gluten, which has the ability to swell from water and retain gases, increasing its volume. Due to these properties of gluten from wheat flour can be obtained bread with a large volume and many pores.

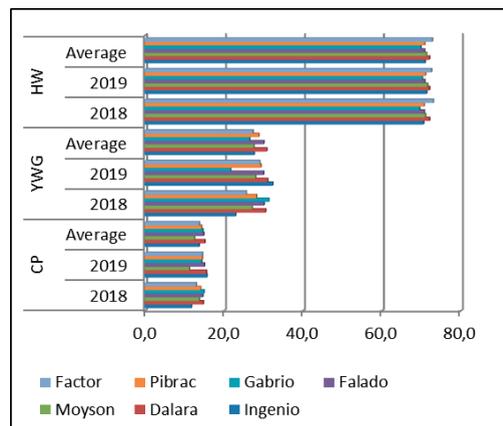


Figure 3. Quality indicators for common wheat varieties (crude protein, wet gluten yield and hectolitre weight) by year

Table 1. Two-factor analysis of variance of the factors: A - year and B - variety on wheat yield

Source of variation	Power of Influence	SS	df	MS	F	P-value	F crit
Year (A) ***	37%	35848256.5	1	35848256.5	273174.8	0.000	4.07
Varieties (B)*	28%	26611475.7	6	4435246	33797.9	0.001	2.32
Interaction (AxB)***	35%	34155786.4	6	5692631.1	43379.6	0.000	2.31
Erroros		5511.6	42	131.2			

\*\*\*, \*\*, \* - proved at  $p \leq 0.001$ ,  $p \leq 0.01$  and  $p \leq 0.05$  respectively; n.s. - unproven

The qualitative composition of the grain is genetically determined, but can change under the influence of growing conditions. The content of nutrients in the soil affects both the amount of wheat yield and the chemical composition of the grain. The yield of wet gluten in the grain provides, on the one hand, the protein content and nutritional value, and on the other hand, guarantees a corresponding amount of gluten in the flour.

Table 2. Summary statistics of the studied quality indicators in common wheat

Indicators	Average value MEAN	Standard deviation SD	MIN	MAX
Crud protein (CP)	14.08	0.90	12.45	15.10
Yield wet gluten (YWG)	28.35	1.57	26.45	30.75
Hectolitre weight (HW)	71.19	0.94	69.85	72.75

average for the period, while in Gabrio it was found -26.5, on average for a two-year period. Over the years, the figure varies for Ingenio from 22.9 to 32.4. Variation was also reported in Gabrio (from 31.6 to 21.6) and Factor (25.6-29.0). The dollar is characterized by a relatively stable 30.5-31.0. Regardless of the environmental conditions, gluten also does not vary in Moyson (27.1-28.0), phalado (30.1-30.0), Pibrak (28.2-29.3). The genetic characteristics of each variety respond differently to environmental conditions. Statistics on the indicators: crude protein, wet gluten yield and hectolitre weight are shown in

Table 1. Crude protein (CP) and hectolitre weight (HW) change in the narrowest interval, and yield wet gluten (YWG).

## CONCLUSIONS

The following conclusions can be drawn from the field study: According to the two-factor analysis of variance, both the influence of the two factors (conditions of the year and variety) separately and their interaction, statistically proven with a very high degree of reliability ( $p \leq 0.001$ ) is the impact on the indicator - yield. The strongest influence on the variation of the trait is exerted by the factor year (37%), followed by the interaction between them (35%) and the factor variety (28%).

In terms of the yield of wet gluten with the highest content, Dalara (30.8) stands out, on average for the period. Climate conditions have a great influence on DMG and it is expressed in the fluctuations of its values in Ingenio (from 22.9 to 32.4), in Gabrio (from 31.6 to 21.6) and in Factor (25.6- 29.0). Despite the changing environmental conditions, a small range of DMG variation was reported in Dalara (30.5 - 31.0), in Moyson (27.1-28.0), in Falado (30.1-30.0), Pibrak (28.2-29.3).

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## COMPARATIVE ASSESSMENT OF SEED MYCOSIS SUSCEPTIBILITY FOR 23 WHEAT CULTIVARS (*Triticum aestivum* L.)

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### Abstract

*Seed microflora is recognised for the influence it can have on crop health and subsequent quantitative and qualitative parameters of the yield. Aim of this study was to determine the mycosis susceptibility of wheat seeds belonging to 23 cultivars obtained from crops that received two fertilization regimes: basic fertilization in autumn and respectively supplementary fertilization in spring in addition to the basic fertilization from autumn. Results showed that seeds from the crop that benefited from supplementary fertilization had a higher average germination rate as well as a frequency decrease in pathogenic fungi. On average the germination rate for wheat was 92.61%, *Alternaria* sp. incidence was 24.67% and *Fusarium* sp. incidence was 21.74%. Cultivars showed heterogeneity regarding frequency of seed infection, suggesting a different susceptibility of the genotypes, but overall, the results obtained for Romanian cultivars were comparable with those for the cultivars from abroad. Results suggest that nutritional status of plants plays a role and supplementary fertilization can ensure healthier seed.*

**Key words:** caryopsis, grains, fungi, germination, incubation.

### INTRODUCTION

Mycoflora of wheat seeds can be rich and complex. Past research has revealed that numerous fungi belonging to various genera can be isolated from wheat seeds, such as: *Alternaria*, *Aspergillus*, *Bipolaris*, *Chaetomium*, *Curvularia*, *Drechslera*, *Epicoccum*, *Fusarium*, *Helminthosporium*, *Myrothecium*, *Nigrospora*, *Penicillium*, *Phoma*, *Rhizopus*, *Mucor*, *Sclerotium*, *Stemphylium* (Toklu et al., 2008; Pathak and Zaidi, 2013; Shad et al., 2019). In general, unsterilized seeds can present pathogenic, saprophytic (Suciu et al., 2020) or endophytic microflora (Hubbard et al., 2012). Phytosanitary condition of seeds, particularly microorganisms load can influence germination or can be responsible for the transmission to the crop of certain diseases (Pathak and Zaidi, 2013; Filatova et al., 2020). However, research has shown that some seed endophytic fungi can enhance resistance of wheat seeds to certain abiotic stress factors (Hubbard et al., 2012), while bacterial endophytes isolated from wheat seeds showed

inhibitory effect against certain pathogenic fungi (Herrera et al., 2016). Latest findings open new prospects for the control of seed-borne pathogens. In wheat crop, seed-borne pathogens are estimated to be responsible for uneven plant development and a yield decrease ranging between 15-90% if infected seeds are sown in the field (Pathak and Zaidi, 2013). Most common pathogenic fungal species colonizing wheat seeds are from genera *Fusarium* and *Alternaria* (Ramires et al., 2018). Fungal seed colonizers from genera such as *Fusarium*, *Alternaria*, *Aspergillus* and *Penicillium* can be responsible for mycotoxin contamination of grains that pose a health threat (Spanic et al., 2020). Preventive strategies dealing with such pathogens have been crop rotation, optimised tillage, straw management, use of genotypes less susceptible to disease (Vogelgsang et al., 2008), fungicide use and other novel pre-sowing seed treatments (Filatova et al., 2020). Because the contamination of seeds with pathogens plays a major role for the success of the crop, the factors that influence the outcome of seed and

crop health are worth investigating due to their practical implications. Aim of this study was to determine the mycosis susceptibility of unsterilized wheat seeds from crops that received two fertilization regimes. Objectives of the study were:

- comparative assessment of germination,
- screening *Alternaria* sp. infection frequency,
- screening *Fusarium* sp. infection frequency.

## MATERIALS AND METHODS

Biologic material for this research was represented by a selection of 11 autochthonous cultivars and 12 cultivars from abroad, that were chosen based on criteria of productivity and stability of production in conditions from Transylvanian Plain, Romania (Table 1). The

seeds used in this study were from the yield of year 2019, from experimental field located at Agricultural Research and Development Station from Turda, Romania. The cultivation followed conventional technology for wheat. The fertilization regime had two variants: V<sub>1</sub> - basic fertilization in autumn with NPK 20:20:0 in doses of 250 kg/ha for ensuring a quantity of 50 kg/ha active substance of Nitrogen as well as Phosphorus; and V<sub>2</sub> - basic fertilization in autumn + supplementary fertilization in spring with 180 kg/ha nitro calcar that ensured 50 kg/ha Nitrogen. For the determination of germination and mycotic susceptibility of the wheat genotypes, seeds were placed for germination on moist filter paper in Petri dishes (Figure 1).

Table 1. Wheat cultivars (*Triticum aestivum* L.) used for screening seed mycosis susceptibility

Provenance	Cultivars
Autochthonous cultivars (Romania)	SCDA Turda: Andrada, Apullum, Arieșan, Codru, Dumbrava, Turda 2000
	INCDA Fundulea: Faur, Miranda, Pitar
	SCDA Suceava: Magistral
	SCDA Lovrin: Alex
Cultivars from abroad	Austria: Capo, Fulvio, Gallio, Josef
	Hungary: Mv Béres, Mv Kolo
	France: Apache, Arlequin, Christine, Element, Renan
	Germany: Exotic

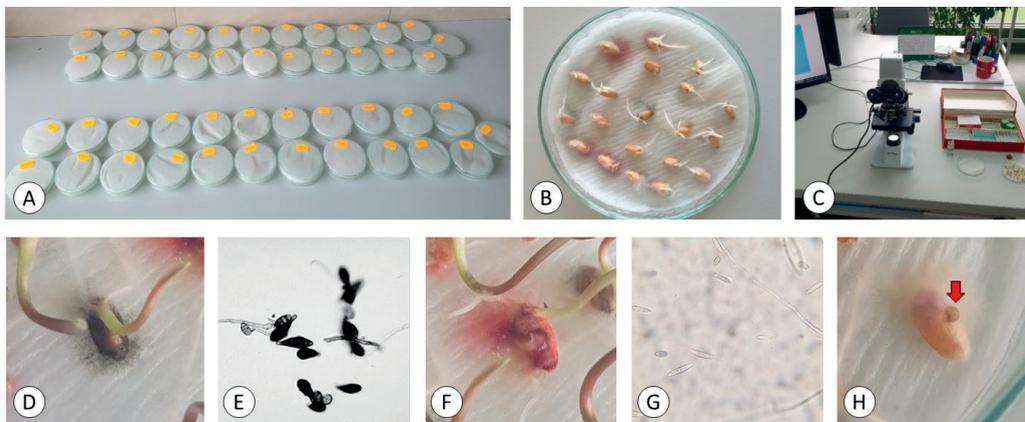


Figure 1. Working protocol and examination of wheat seedlings: a) Petri dishes with seeds during incubation, b) Petri dish after 4 days of incubation, c) microscopic analysis setup, d) macroscopic aspect of *Alternaria* sp. infection, e) microscopic observation of *Alternaria* sp., f) macroscopic aspect of *Fusarium* sp. infection, g) microscopic observation of *Fusarium* sp., h) arrow - bacterial exudate (original)

The Petri dishes were at first disinfected with alcohol and then water-soaked filter paper was placed in each. The Petri dishes with seeds

were maintained closed at room temperature. After four days were conducted the macroscopic and microscopic analyses (Figure

1). Germination rate, and incidence of *Alternaria* sp. as well as *Fusarium* sp. was calculated as frequency (%) of germinated seeds from the number of seeds placed at incubation – in the case of each experimental variant.

## RESULTS AND DISCUSSIONS

Average germination rate for all cultivars regardless of fertilization regime was 92.61%, ranging between 80-100%. Also, average germination rate per cultivar was  $\geq 90\%$  in 18 out of 23 cultivars studied. Average germination rate associated with supplementary fertilization was 93.26%, while average germination rate associated with basic fertilization alone was lower (91.96%). The improvement brought by supplementary fertilization of the crop on germination rate of seeds was observed both for Romanian cultivars as well as for cultivars from abroad. Thus, average germination rate for the seeds of Romanian cultivars from the crop that benefited only of the basic fertilization ( $V_1$ ) was 92.27%, while germination rate for seeds from the crop that enjoyed supplementary

fertilization ( $V_2$ ) increased to 93.64%. Similarly, the average germination rate for cultivars from abroad was 91.67% associated with basic fertilization alone ( $V_1$ ), and 92.92% when supplementary fertilization was used for the crop ( $V_2$ ). Two Romanian cultivars ('Arieșan', 'Dumbrava') as well as one cultivar from abroad ('Apache') had a germination rate of 100% regardless of fertilization regime of the crop (Figure 2). Supplementary fertilization increased the germination rate of the seeds to 100% for cultivars 'Alex' and 'Christine'. Lowest germination rate for seeds obtained from the plot that benefited only of basic fertilization ( $V_1$ ) was identified for Romanian cultivar 'Pitar' (75%) and French cultivar 'Renan' (65%). But the same cultivars under supplementary fertilization had a germination  $\geq 90\%$ , indicating that fertilization can maximize the potential of these cultivars. Lowest germination rate for the seeds from the crop that benefited from supplementary fertilization besides basic fertilization ( $V_2$ ) was identified in three cultivars: Romanian cultivar 'Codru' as well two cultivars from abroad 'Josef' and 'Mv Béres' - all three having a germination rate of 85% (Figure 2).

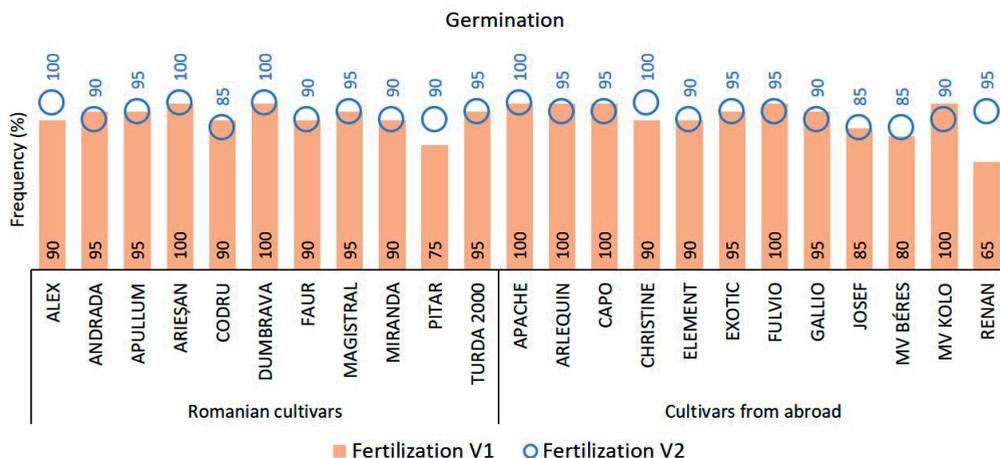


Figure 2. Germination frequency (%) for 23 wheat cultivars from crops with two fertilization regimes:  $V_1$ - basic fertilization in autumn,  $V_2$ - basic fertilization in autumn + supplementary fertilization in spring

Macroscopic and microscopic analysis of seeds after incubation allowed the identification of fungal pathogens (Figure 1d, 1e, 1f, 1g). Some seeds showed also signs of bacterial infection, observed based on bacterial exudate (Figure

1h) but overall incidence was low, less than 5%. *Alternaria* sp. infection (Figure 1d, 1e) presented an overall incidence of 24.67%, with a range between 5-40%. Seeds from the crop that had only basic fertilization had overall a

higher *Alternaria* sp. incidence of 25.22%, compared to seeds from the crop that benefited from supplementary fertilization (24.13%). This trend was observed both for average incidence in Romanian cultivars as well as for average incidence in cultivars from abroad. Thus, on average the seedlings of the Romanian cultivars had an *Alternaria* sp. incidence of 27.27% associated with basic fertilization regime (V<sub>1</sub>), and this decreased to 25.45% for seeds from crop that benefited also of supplementary fertilization (V<sub>2</sub>). Similarly, the average *Alternaria* sp. incidence for the cultivars from abroad was 23.33% corresponding to the crop with basic fertilization alone (V<sub>1</sub>) and decreased to an average of 22.92% due to supplementary fertilization of the crop (V<sub>2</sub>). Highest level of *Alternaria* sp. infected seedlings for seeds from

the crop that benefited only of basic fertilization was 35%, and identified in cultivars ‘Dumbrava’, ‘Faur’, ‘Turda 2000’, ‘Exotic’ and ‘Mv Béres’ (Figure 3). Seeds from the crop that benefited from supplementary fertilization had a maximum incidence of 35% identified in Romanian cultivars ‘Arieșan’ and ‘Codru’, while French cultivar ‘Apache’ reached a level of 40% seeds affected by *Alternaria* sp. (Figure 3). Lowest frequency of *Alternaria* sp. (≤15%) was identified for cultivars ‘Andrada’, ‘Christine’, ‘Fulvio’, ‘Gallio’, when seeds were from the crop that had only basic fertilization (V<sub>1</sub>). When crop enjoyed supplementary fertilization, the seeds of the cultivars ‘Alex’, ‘Miranda’, ‘Capo’, ‘Christine’, ‘Gallio’ had a low incidence of *Alternaria* sp. (Figure 3).

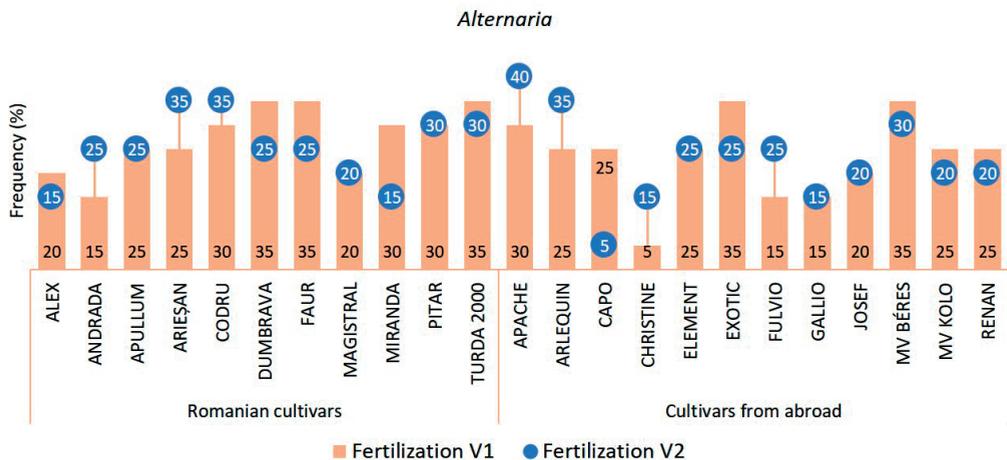


Figure 3. Frequency (%) of *Alternaria* sp. infection for 23 wheat cultivars from crops with two fertilization regimes: V<sub>1</sub> - basic fertilization in autumn, V<sub>2</sub> - basic fertilization in autumn + supplementary fertilization in spring

*Fusarium* sp. infection (Figure 1f, 1g) presented an overall incidence of 21.74%, with a range between 10–40%. Seeds from the crop that benefited only from basic fertilization had overall a higher *Fusarium* sp. incidence (22.39%), compared to seeds from the crop that benefited from supplementary fertilization (21.09%). This trend was observed for average incidence in Romanian cultivars as well, but not for the average incidence in cultivars from abroad. Thus, on average the seedlings of the Romanian cultivars had a *Fusarium* sp. incidence of 24.55% under basic fertilization

alone (V<sub>1</sub>), and this decreased to 20.45% for seeds from the crop that also had supplementary fertilization (V<sub>2</sub>). By comparison, the average *Fusarium* sp. frequency of the cultivars from abroad was 20.42% for seeds from the crop with basic fertilization alone (V<sub>1</sub>) and increased to an average of 21.67% for seeds from the crop that enjoyed also a supplementary fertilization (V<sub>2</sub>). Highest frequency of *Fusarium* sp. infection (40%) of seedlings corresponding to seeds from crops that received only basic fertilization was observed for cultivars ‘Dumbrava’ and ‘Mv

Béres'. Highest frequency of *Fusarium* sp. infection of seedlings corresponding to seeds from the crop that enjoyed supplementary fertilization was 35% and occurred in cultivar 'Miranda'. Lowest *Fusarium* sp. incidence ( $\leq 15\%$ ), was observed for cultivars 'Alex', 'Faur', 'Magistral', 'Turda 2000', 'Apache',

'Element', 'Exotic', 'Fulvio', 'Gallio', when seeds were from the crop that had only basic fertilization. When seeds were from the plot that received supplementary fertilization, the lower frequency occurred in cultivars 'Andrada', 'Arieşan', 'Faur', 'Magistral', 'Apache', 'Exotic', 'Gallio' (Figure 4).

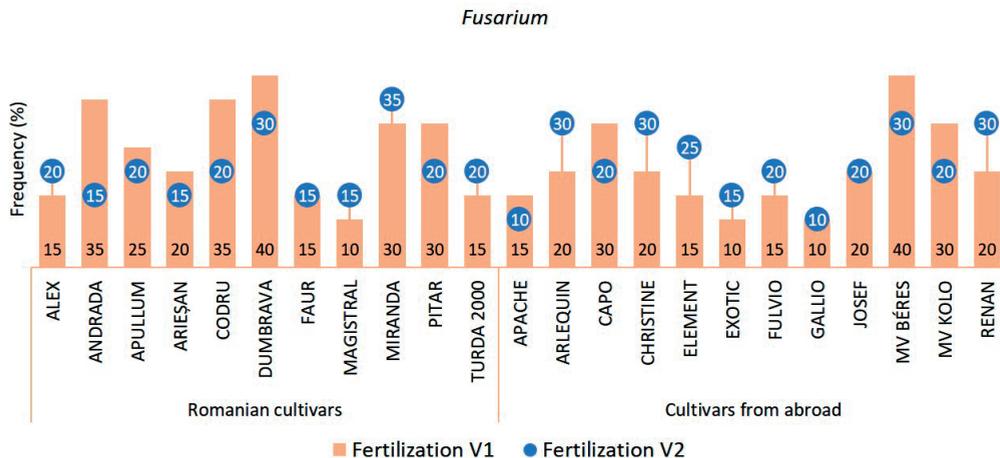


Figure 4. Frequency (%) of *Fusarium* sp. infection for 23 wheat cultivars from crops with two fertilization regimes: V<sub>1</sub>- basic fertilization in autumn, V<sub>2</sub>- basic fertilization in autumn + supplementary fertilization in spring

Results of the present research suggests that fertilization regime can have an influence both on the germination capacity as well as on mycotic load of the seeds. Thus, seeds that were from the plot that received besides the basic fertilization in autumn also a supplementary fertilization in spring, presented overall a higher germination rate as well as lower *Fusarium* sp. and *Alternaria* sp. infection incidence in seeds. This further infers that nutritional status of the plant can influence the seed quality as well as mycotic load of the seeds. Regardless of fertilization regime, several cultivars displayed a maximum germination rate ('Arieşan', 'Dumbrava', 'Apache') or a lower frequency for *Alternaria* sp. ('Christine', 'Gallio'), and *Fusarium* sp. ('Faur', 'Magistral', 'Apache', 'Exotic', 'Gallio').

Globally, the most serious and hazardous disease of wheat is *Fusarium* head blight disease (FHB) (Figuroa et al., 2018). In Europe, FHB is caused primarily by *Fusarium graminearum*, *F. culmorum*, *F. avenaceum* and *F. poae* (Vogelgsang et al., 2008), and is

associated with mycotoxin contamination of wheat grains. Although *Alternaria* spp. represents also a highly common occurring fungi on seeds worldwide, there are some conflicting views on the harmfulness of disease, and this is most likely due to diversity of this genus. *Alternaria* spp. found in seeds can be either saprotrophic, slightly phytopathogenic and is either producing or not producing mycotoxins (Gannibal, 2018). Frequent colonizers of wheat seeds are the species *Alternaria alternata*, *A. arborescens*, *A. infectoria*, *A. tenuissima*, and *A. triticina* (Ramires et al., 2018). There is evidence that infected seeds and seed transmission is the main source of infection with *Alternaria* sp. in wheat crops (Perelló and Larrán, 2013). Most often wheat kernels infected by *Alternaria* species are characterized by black-spot pigmentation in the underlying embryo region. This feature is associated with low quality of the flour and potential health risk due to some harmful fungal metabolites (Ramires et al., 2018).

Similar to the results of this study, previous research conducted in various countries, also

identified *Alternaria* sp. and *Fusarium* sp. among the most frequent seed-borne pathogenic fungi of wheat. Thus, a study in Turkey revealed that *Alternaria* had highest occurrence in wheat seeds regardless whether kernels placed for germination displayed black-point pigmentation, discoloration or had healthy aspect (Toklu et al., 2008). Another study on wheat seeds from Lithuania revealed that samples were colonized by *Alternaria*, *Fusarium* and *Penicillium* besides a few other fungi genera (Sinkevičienė and Šaluchaitė, 2020). In wheat samples from Argentina, *A. tenuissima* was the dominant fungi from genus *Alternaria* colonizing the seeds, followed by *A. infectoria*, *A. triticimaculans*, *A. triticina*, *A. alternata* and *A. chlamydospora* besides a few other related fungi (Perelló and Larrán, 2013). In Russian wheat, the frequency of *Alternaria tenuissimais* and *A. infectoria* was shown to reach levels up to over 70% (Gannibal, 2018). Organically grown wheat from Italy, had average seed infection rate less than 5% for either of the several *Fusarium* species identified, but *Fusarium poae* was one of the most abundant (Infantino et al., 2011). A long-term trial conducted in Switzerland also identified *F. poae* as the most prevalent *Fusarium* species in wheat seeds (Vogelgsang et al., 2008). As for the location of the pathogen, this was determined to be in seed coat of wheat for *Fusarium avenaceum* and *F. poae*, while *F. graminearum* was identified in seed coat and embryo (Hassani et al., 2019). By comparison, in cereal crops only the seed coat is affected by *Alternaria* infection, and the embryo can germinate, and under favourable conditions the seedling can survive and grow (Gannibal, 2018). One question is whether different fungi species from same genus that cause similar symptomatology could have a variable influence on seed germination. In this sense, Browne and Cooke (2005) succeeded to put in evidence the influence of infection with several *Fusarium* species on the average wheat seed germination relative to control with following results: *F. graminearum* (61.7%), *F. avenaceum* (65.5%), *Fusarium culmorum* (76.6%), *F. poae* (92.5%). Furthermore, some species of *Fusarium* appear to be more aggressive, since isolate mixture of *F. avenaceum* showed higher pathogenicity

than *F. poae*, causing higher disease levels as well as higher toxin accumulation in wheat grains (Vogelgsang et al., 2008). Presence of the pathogens on seed is associated with both seed quality and safety (Ramires et al., 2018). An experiment proved that low levels of *Fusarium graminearum* seed infection was associated with higher wheat seed quality and significant positive correlation (0.24\*) was found between seed infection and mycotoxin (deoxynivalenol) content (Argyris et al., 2003). Interesting perspectives have been identified regarding the potential treatments of wheat seeds against common fungal pathogens, as pre-sowing measures. Thus, out of 600 plant-associated bacterial isolates (e.g. *Pantoea* sp., *Pseudomonas* sp.), 16% isolates showed over 80% *Fusarium culmorum* disease suppression. Efficacy in this case was influenced by bacterial dose and suspension volume (Johansson et al., 2003). Same direction was explored by more recent authors that demonstrated that bacterial isolates (*Pantoea* sp., *Paenibacillus* sp.) from wheat seeds inhibited *F. graminearum* colonies on agar plates, with reliable results on seeds following same treatment (Herrera et al., 2016). Because crop rotation is often the recommended practice to reduce the inoculum source of fungal pathogens in wheat, some authors investigated whether there are potential alternative inoculum sources. Their results showed that *F. graminearum* isolated from wheat seeds, caused decreased germination energy in pea and lupine seeds with potential implications for crop rotation (Rasiukeviciute and Kelpsiene, 2018). It has been asserted that environmental conditions favour the growth and spread of fungi, that can ultimately compromise seed quality (Gannibal, 2018; Shad et al., 2019). But so far, influence of other factors such as fertilization regime on microorganism load were insufficiently explored. In this sense the present research provides a preliminary path for future investigations on the role of nutritional status of the plants from different genotypes on pathogenic fungi occurrence on seeds.

## CONCLUSIONS

This research investigated the frequency of germination as well as *Fusarium* and

*Alternaria* presence on seeds for 23 wheat cultivars.

Results showed that average germination rate was 92.61%, overall *Alternaria* sp. incidence in seeds was 24.67% and overall *Fusarium* sp. incidence was 21.74% for seeds of 2019 crop obtained in conditions from Transylvanian Plain. Analysis showed that seeds from the crop that benefited from supplementary fertilization in spring besides basic fertilization in autumn, had an increase of average germination from 91.96% to 93.26%, as well as a decrease of pathogenic fungi incidence. Thus, average *Alternaria* sp. incidence decreased from 25.22% associated with basic fertilization alone, to 24.13% for seeds from the crop that benefited also of supplementary fertilization. Similarly, average *Fusarium* sp. incidence decreased from 22.39 % associated with basic fertilization to 21.09%. Cultivars displayed heterogeneity regarding incidence of seed infection associated with fertilization regimes, suggesting a different susceptibility of the genotypes as well as a potential influence of nutritional status of plants during vegetation on mycotic load of the seeds.

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## RESEARCH ON THE INFLUENCE OF TECHNOLOGICAL LINKS ON OIL RAPESEED PRODUCTION

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### **Abstract**

*Research carried out in the Western Plain of Romania, on a soil of carbonate chernozem type, wet, weakly salinized, in sodium depth, aimed to make contributions to the oil rapeseed cultivation technology, a culture particularly important for the area, on optimizing nitrogen fertilization and determining the type of fertilizer - ammonium nitrate or ammonium sulphate - for the above mentioned soil conditions, under the conditions of fattening a constant phosphorus and potassium background ( $P_{80}K_{80}$ ), as well as the influence on production according to the date of operation and depth of the basic ploughing. The results showed that the application of nitrogen fertilizers in the form of ammonium sulphate was superior to the one of ammonium nitrate by 8%. Also, the results according to the period of execution of the basic ploughing emphasize the importance of carrying out this important work as early as possible, so that by the date of sowing to sit down and accumulate as much water in the soil.*

**Key words:** nitrogen fertilization, Timis Plain, Romania.

### **INTRODUCTION**

Due to the widespread use of rapeseed and for the purpose of producers of biodiesel and bioethanol, it is observed or continuous growth of cultivated areas (Fridrihsone et al., 2020; Zhang et al., 2013).

Oil rapeseed is a crop that reacts very well to mineral fertilization, but it is very important in addition to determining the time of application, also the correlation of the type of fertilizer, according to the type of soil and especially the soil reaction (Haneklaus et al., 1999; Wang et al., 2010; Matei et al., 2017; Louvieux et al., 2020; Men et al., 2020).

Climate change in recent times also has a detrimental influence on rapeseed production, especially in very dry autumns when the percentage of plants rising is very low (Bătrâna, et al., 2020; Eyni Nargeseh et al., 2020).

In the current context, characterized by long periods of drought, the establishment of autumn rapeseed crops can raise many problems related to the performance of the ploughing, the establishment of the depth and the period of execution, in order to combat weeds on one hand, and on the other one, to favour the

accumulation of as much water in the soil as possible, as well as to preserve the existing moisture in the soil favouring a good growth of the culture (Grosz et al., 2011; Şmuleac et al., 2020; Bečka et al., 2021).

### **MATERIALS AND METHODS**

The research carried out in the salinized soils area had the following goals: to determine the efficiency of the doses and of the types of nitrogen fertilizer used in the salinized soil area on oil rapeseed cultures; to do research regarding the influence of the execution period, of the ploughing depth and of the salinized soil area on the oil rapeseed yield.

In order to find an answer to these questions the cultures have been organized in the Timiș Plain, on a wet phreatic carbonate chernozem, poorly salinized in its depth. The experiments have been bifactorial and there have been done three repetitions. The experiments done based on the nitrogen fertilization were:

A Factor – the type of nitrogen fertilizer used:  
a<sub>1</sub> - ammonium nitrate; a<sub>2</sub> - ammonium sulphate.

B Factor - the doses of nitrogen applied on a constant base of  $P_{80}K_{80}$ : b<sub>1</sub> - N<sub>0</sub>; b<sub>2</sub> - N<sub>50</sub>; b<sub>3</sub> -

N<sub>100</sub>; b<sub>4</sub> - N<sub>150</sub>; b<sub>5</sub> - N<sub>200</sub>. There have been done research regarding the settlement of the date on which the ploughing should be done and of the ploughing depth for the crops and there have been organized bifactorial experiments with three repetitions.

The factor graduations were the following: A Factor - the ploughing date: a<sub>1</sub> - 10-15 July; a<sub>2</sub> - 10-15 August; a<sub>3</sub> - 10-15 September.

B Factor - the ploughing depth: b<sub>1</sub> - 18-20 cm; b<sub>2</sub> - 23-25 cm; b<sub>3</sub> - 28-30 cm.

## RESULTS AND DISCUSSIONS

Synthesis data on the harvests obtained according to the doses of fertilizers used, the yield obtained were between 992 kg/ha (N<sub>0</sub>) and 4537 kg/ha (N<sub>200</sub>), when fertilization was done with ammonium nitrate and between 992 kg/ha (N<sub>0</sub>) and 4716 kg/ha (N<sub>200</sub>) when fertilization was done with ammonium sulphate (Table 1).

On an average, on the applied nitrogen doses, by using the ammonium sulphate, conducted to a harvest increase of 5%, resulting in a significant difference of 179 kg/ha.

The harvest differences according to the nitrogen fertilizer dose applied, on an average on the two types of fertilizer, were of 1300 kg/ha in the variant fertilized with N<sub>50</sub>, 2362 kg/ha in the fertilized variant with N<sub>100</sub>, 2898 kg/ha in the fertilized variant with N<sub>150</sub> and 3635 kg/ha, in the fertilized variant with N<sub>200</sub>.

One may notice that all crop differences, depending on the nitrogen dose, are ensured as very significant.

The results lead to the conclusion that rapeseed for oil under the conditions of carbonate

chernozem, poorly salinized, capitalize very good the nitrogen fertilizers.

Synthesis data on the evolution of the oil content highlight the negative influence of nitrogen fertilizers that caused the content's decrease, together with the increase in doses (Figure 1).

In the researched field, with the use of ammonium nitrate the decreased oil content was from 42.3 (N<sub>0</sub>) to 38.8% (N<sub>200</sub>), and in the fertilized variants with ammonium sulphate the decrease was 42.3% (N<sub>0</sub>) to 39.5% (N<sub>200</sub>). It results that in the use of ammonium nitrate the decrease of the oil content was 0.7% higher (at the N<sub>200</sub> dose level) compared to the use of the same dose but under the form of ammonium sulphate.

Oil production points out that in the researched area N<sub>0</sub> - N<sub>200</sub> oil production ranged from 420 kg/ha to 1760 kg/ha when the application was made under the form of ammonium nitrate and between 420 kg/ha and 1863 kg/ha, when the application was made in the form of ammonium sulphate (Table 2). On an average, on the applied doses, oil production was 6% higher when the application was made under the form of ammonium sulphate, compared with the one under the form of ammonium nitrate application.

In conclusion, nitrogen fertilisers negatively influenced the seed oil content due to the favourable effect on the crop and have determined the increase of oil production with very significant differences towards the control (N<sub>0</sub>) by 536 kg/ha (N<sub>50</sub>), 942 kg/ha (N<sub>100</sub>), 1121 kg/ha (N<sub>150</sub>) and 1392 kg/ha (N<sub>200</sub>).

Table 1. The syntheses of the yield results obtained for the oilseed rape according to the type of the used nitrogen fertilizer and to the used fertilizer doses

Factor A Type of fertilizer	Factor B - Nitrogen doses on the merits of P <sub>30</sub> K <sub>80</sub>					Average Factor A			
	No <sub>0</sub>	No <sub>50</sub>	No <sub>100</sub>	No <sub>150</sub>	N <sub>200</sub>	Crop (kg/ha)	%	Difference (kg/ha)	Significance
Ammonium nitrate	992	2172	3240	3824	4537	2953	100		
Ammonium sulfate	992	2412	3467	3955	4716	3108	105	179	X

DL 5% = 168 kg/ha; DL 1% = 210 kg/ha; DL 0.1 % = 273 kg/ha

Average Factor B

Specification	No <sub>0</sub>	No <sub>50</sub>	No <sub>100</sub>	No <sub>150</sub>	N <sub>200</sub>
Crop kg/ha	992	2292	3354	3890	4627
%	100	231	338	392	466
Difference		1300	2362	2898	3635
Significance		XXX	XXX	XXX	XXX

DL 5% = 191 kg/ha DL 1% = 252kg/ha DL 0.1% = 327 kg/ha

Table 2. The syntheses of the oil production results obtained for the oilseed according to the used type of nitrogen fertilizer and to the experimented fertilizer doses

Factor A Type of fertilizer	Factor B - Nitrogen doses on the merits of P <sub>80</sub> K <sub>80</sub>					Average Factor A			
	No <sub>0</sub>	No <sub>50</sub>	No <sub>100</sub>	No <sub>150</sub>	N <sub>200</sub>	Crop (kg/ha)	%	Difference (kg/ha)	Significance
Ammonium nitrate	420	904	1312	1507	1760	1180			
Ammonium sulfate	420	1008	1411	1574	1863	1255	106	75	

DL 5% = 83 kg/ha; DL 1% = 127 kg/ha; DL 0,1 % = 164 kg/ha

Average Factor B

Specification	No <sub>0</sub>	No <sub>50</sub>	No <sub>100</sub>	No <sub>150</sub>	N <sub>200</sub>
Crop kg/ha	420	956	1362	1540	1811
%	100	228	324	367	324
Difference		536	942	1121	1392
Significance			XXX	XXX	XXX

DL 5% = 87 kg/ha; DL 1% = 123kg/ha; DL 0,1 % = 176 kg/ha

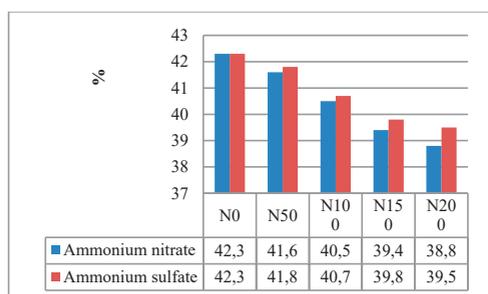


Figure 1. The rapeseed oil content variation according to the type of nitrogen fertilizer and to the applied fertilizer doses

The results obtained according to the depth of the ploughing and the date of its completion underline the importance of carrying out this important work as early as possible, so that by the date of sowing the land settles and accumulates as much water in the soil (Table 3). Compared to the ploughing carried

out in mid-July, by delay of one month, the harvest decreased by 12% namely by 348 kg/ha. The delay in the completion date until the end of August increased crop losses by 28%, resulting in a decrease of 794 kg/ha.

The increase in the depth of ploughing execution from 18-20 cm to 23-25 cm increased the harvest by 11%, returning a difference of 238 kg/ha. Increasing the depth of the work to 28 to 30 cm contributed to the increase of the harvest by 21%, respectively by 477 kg/ha.

The results obtained show that the date of execution of the ploughing and the depth of its performance to the limit where there is no danger of bringing to the surface the layer with higher concentration of salts, represents for rapeseed an important link in the chain of cultivation technology.

Table 3. The syntheses of the results obtained for the oilseed rape in according to the date and to the depth of the basic ploughing

Factor A Date	Factor B - Depth (cm)			Average Factor A			
	18 - 20	23 - 25	28 - 30	Crop (kg/ha)	%	Difference (kg/ha)	Significance
10.07 – 15.07	4593	4872	5049	4838	100		
10.08- 15.08	4295	4468	4706	4490	88	- 348	0
15.08 – 30.08	3770	4030	4333	4044	72	- 794	000

DL5% = 214 kg/ha DL 1% = 354 kg/ha DL 0.1% = 649 kg/ha

Average Factor B

Specification	18 - 20	23 - 25	28 - 30
Crop (kg/ha)	4219	4457	4696
%		111	121
Difference (kg/ha)		238	477
Significance		XXX	XXX

DL 5% = 58 kg/ha DL 1% = 82 kg/ha DL 0.1% = 115 kg/ha

## CONCLUSIONS

The application of nitrogen fertilisers in the form of ammonium nitrate and ammonium sulphate with doses of N<sub>50</sub>-N<sub>200</sub> on the background of P<sub>80</sub>K<sub>80</sub> provided harvest increases between 70% (N<sub>50</sub>) and 466% (N<sub>200</sub>).

The application of nitrogen fertilisers in the form of ammonium sulphate was superior to the application in the form of ammonium nitrate by 5% and with a significant difference of 179 kg/ha.

The rapeseed oil content ranged from 42.3% (N<sub>0</sub>) to 38.8% (N<sub>200</sub>) when the ammonium nitrate was used also between 42.3% (N<sub>0</sub>) and 39.5% (N<sub>200</sub>) when ammonium sulphate was used.

Although nitrogen fertilizers negatively influenced the rapeseed oil content, intended for the favourable effect on the seed harvest, they increased oil production with very significant differences towards the control, by 536 kg/ha for the N<sub>50</sub>, by 942 kg/ha for the N<sub>100</sub>, by 1121 kg/ha for the N<sub>150</sub> and by 1392 kg/ha for the N<sub>200</sub> fertilised variants.

The date of the autumn rapeseed ploughing is of a particular importance given the early sowing of this crop. The delay of the ploughing effect until the end of August results in harvest losses of 794 kg/ha.

The depth of execution of the ploughing up to 28-30 cm, the depth to which there is no danger of bringing the layer with high concentration of salts, determined the growth by 21% of the harvest, compared to a ploughing of 18-20 cm.

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## INFLUENCE OF THE TREATMENT SCHEME ON THE CERCOSPORIOSIS ATTACK (*Cercospora beticola* Sacc.)

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### Abstract

Researches were carried out in the experimental field from the locality Sanzieni, Covasna county, with the aim of determining the effectiveness of some treatment schemes in combating the pathogen *Cercospora beticola* Sacc. of the sugar beet. The biological material was represented by the Damian, Matti, Vangelis and Tetry varieties. Different treatment schemes were tested and efficacy was calculated. The treatment reduced significantly the attack of cercosporiosis in all the analyzed varieties. The Damian variety registered the highest efficacy value of 91.27% in the Flowbrix + Amistar Gold + Sphere 535 Sc + Solar Bor treatment scheme. The application of treatment schemes led to statistically guaranteed production increases.

**Key words:** sugar beet, treatment, cercosporiosis, efficacy.

### INTRODUCTION

Sugar beet (*Beta vulgaris* L.) represents one of the most important agricultural plants grown for sugar production in Romania and in many countries around the world (Toth & Cristea, 2018). Leaf spot disease or cercosporiosis is caused by the attack of the mycomycete *Cercospora beticola* Sacc. Cercosporiosis is one of the most common and destructive diseases of sugar beet (Skaracis et al., 2010; Cristea, 2005), with a major impact on root production and sugar content (Smith & Martin, 1978). An integrated control strategy of the attack of this pathogen takes into account the reaction of varieties (Rosi, 1995), proper rotation, cultural hygiene, fungicide treatments paying attention in their application to the resistance of the fungus *Cercospora beticola* to the administered substances (Wolf & Verreet, 2002; Shane & Teng, 1992; Dovas et al., 1976; Meriggi et al., 2000). In controlling the attack of the fungus *Cercospora beticola* it is necessary to take into account all the factors involved in the onset and evolution of the disease and in the use of treatment schemes that will ensure a high effectiveness on root production (Toth & Cristea, 2020). Given the importance and implications of the attack of cercosporiosis in sugar beet culture,

comprehensive studies have recently been conducted on the disease, the involved pathogen and on dealing with its attack. (Asher et al., 2000).

### MATERIALS AND METHODS

The research was carried out in 2020 in the experimental field within SC Sinzieni, Covasna County, and consisted in monitoring the influence of some fungicide treatment schemes recommended in combating the pathogen *Cercospora beticola*. The biological material used was represented by the monogerm varieties: Damian, Matti, Vangelis and Tetry. The experiments were placed in three repetitions according to the randomized block scheme. The seed of the monitored hybrids was treated with Cruiser 600 products at a dose of 60 g/UG (insecticide) and Tachigaren at a dose of 10 g/UG (fungicide). In the treatment schemes consideration was given to factor 1-variety, factor 2-fungicide and factor 3-fertilizer. Observations were made on frequency (F%) and intensity (I%) of the attack, on which the attack rate was calculated (GA%) The following formulas were used:  $F = nx100/N$ , where N = number of plants observed (%), n = number of plants characteristic symptoms (%),  $I = \sum (ixf)/n$  (% 0 where, i =

provided percentage,  $f$  = number of plants (organs) with the respective percentage,  $n$  = total number of attacked plants (organs). Intensity was marked in percentage. The degree of attack was calculated according to the formula: DA (degree of attack) =  $F \times I/100$  (%), where:  $F$  = attack frequency (%),  $I$  = attack intensity (%), DA = rate was calculated by:  $DA = F \times I/100$  (%), where:  $F$  = attack frequency (%),  $I$  = attack intensity (%). The efficacy of the treatments was calculated by the Abbott's formula:  $E = [(GAM-GAV)/GAM] \times 100$ , where  $GAM$  = control level,  $GAV$  = variant attack level. Data regarding the obtained productions were statistically processed by the analysis of the variant.

## RESULTS AND DISCUSSIONS

The three experiments were located in the experimental field in Sînzieni, Covasna County, in order to monitor the influence of some fungicides that were subject of some treatment schemes in controlling the

micromycete *Cercospora beticola* at sugar beet, in 2020.

The applied scheme also includes a product with contact action, Flowbrix and two products with systemic action, Sfera 535 SC and Amistar Gold. The treatment scheme also includes the applied fertilizer (Table 1). The application of boron reduces the attack of sugar beet specific diseases (Lazar et al., 1977).

The data regarding the observations made on the influence of the treatment scheme on the attack of the micromycete *Cercospora beticola* in the conditions of the year 2020, show that the highest attack frequency value ( $F = 38\%$ ) was registered at the Vangelis variety in the third experiment, and the lowest value of its incidence determined in the third treatment experience ( $F = 25\%$ ) in the Matti variety. For the Vangelis and Tatry varieties a frequency value of 37% was determined under the conditions of the first experiment. High values of frequency ( $F = 37\%$ ) were recorded by the Vangelis variety in the case of the second experiment.

Table 1. Application Scheme of Treatments to Sugar Beet in Controlling the Attack of *Cercospora beticola* at SC Agromiki SRL, Location Sînzieni, Covasna County, 2020

Experiment 1/Scheme I				
Treatment No.	Product	Active substance	Dose (l/ha)	Date/Period of Application
1	Sfera 535 SC	trifloxystrobin 375 g/l + cyproconazole 160 g/l	0.5	July 6
	Wuxal macromix	NPK	1.0	
2	Flowbrix	copper metal in the form of copper oxychloride 380g/l	2.5	July 25
	Solar Bor	15% Bor	2.0	
3	Amistar Gold	azoxystrobin 125g/l + difenoconazole 125g/l	1.0	August 27
	Solar Bor	15% Boron	2.0	
Experiment 2/Scheme II				
1	Amistar Gold	azoxystrobin 125g/l + difenoconazole 125g/l	1.0	July 6
	Yara	3,1%N, 34,3% K2O	2.0	
2	Sfera 535 SC	trifloxystrobin 375 g/l + cyproconazole 160 g/l	0.5	July 25
	Solar Bor	15% Boron		
3	Flowbrix	copper metal in the form of copper oxychloride 380g/l	2.5	August 27
	Solar Bor	15% Boron		
Experiment 3/Scheme III				
1	Flowbrix	copper metal in the form of copper oxychloride 380g/l	2.5	July 6
	Wuxal macromix	NPK	2.0	
2	Amistar Gold	azoxystrobin 125g/l + difenoconazole 125g/l	1.0	July 25
3	Sfera 535 SC	trifloxystrobin 375 g/l + cyproconazole 160 g/l	0.5	August 27
	Solar Bor	15% Boron	2.0	

The Matti variety registered low attack frequency values in the other variants of the treatment scheme, as well, with attack frequency values of cercosporiosis of 28% in the second experiment and respectively 29% in the case of the first experiment. In the case of the Damian variety, the lowest value of the leaf

attack frequency was noted in the case of experiment three, where the value was  $F = 28\%$ . In the second and third experiments on the Damian variety the incidence of the attack had values close to 31% and 33%. The frequency of the attack of cercosporiosis in the Tatry variety showed a high level between 35%

in the second experiment, 36% in the third experiment and 37% in the first experiment. The frequency values in the control variant amounted to 92% for the Vangelis variety and to 89% for the Tatry variety (Table 2).

Table 2. Influence of Treatments Scheme on the Attack of *Cercospora beticola* Sacc. on Sugar Beet at SC Agromiki SRL, Location Sânzieni, Covasna County, 2020

Experiment/Witness	Frequency(F%) Intensity(I%) Degree of attack (DA%)	Variety			
		Damia n	Matt i	Vangeli s	Tatr y
Experiment 1	Frequency	31	29	37	37
	Intensity	3.6	2.4	3.8	3.2
	Degree of attack	1.11	0.69	1.40	1.18
Experiment 2	Frequency	33	28	37	35
	Intensity	3.2	2.1	3.1	2.7
	Degree of attack	1.05	0.58	1.14	0.94
Experiment 3	Frequency	28	25	38	36
	Intensity	2.1	3.1	3.1	2.1
	Degree of attack	0.58	1.17	1.17	0.75
Control	Frequency	87	81	92	89
	Intensity	6.5	5.8	7.2	6.7
	Degree of attack	6.65	4.69	6.62	5.96

As for the observations regarding the attack intensity of *Cercospora beticola* Sacc. the data from the same table show that the values were remarkably lower than those of the attack frequency. The lowest intensity values were calculated for the Damian and Tatry varieties in treatment scheme three, at I = 2.1%. For the Vangelis variety, the intensity of the attack had the same value in the second and third experiments in the treatment scheme, I = 3.1%. A reduced value of intensity was also calculated for the Matti variety in the case of the second experiment (I = 2.1%) (Table 2). Regarding the degree of attack, it was found that its subunit values were determined for the Matti varieties following the application of the treatments from the first and second experiments in the scheme (DA = 0.69% and DA = 58%) for the Tatry variety in the second (DA = 0.94%) and third (DA = 0.75%) experiment. The Damian variety recorded a minimum value of the degree of attack of DA = 0.58% in the third experiment. The highest values were calculated in the control variant for the Vangelis varieties (DA = 6.62%) and for

the Damian variety (DA = 6.65%). Research shows that the application of fungicides has significantly reduced the attack of cercosporiosis in varieties monitored in the experimental area (Toth & Cristea, 2020). Application of fungicide treatments for controlling the pathogen *Cercospora beticola* Sacc. is one of the important interventions in controlling the sugar beet pathogen. Research has shown that the application of treatments is effective in controlling cercosporiosis (Toth & Cristea, 2020). Sugar beet cercosporiosis control strategy considers the genotype and control by using approved fungicides (Skaracis et al., 2010; Cristea 2005). The calculation of the effectiveness of the treatments applied in the experimental variants was taken into consideration (Table 3).

Table 3. Efficacy on Attack of *Cercospora beticola* on Sugar Beet, Location Sânzieni, Covasna County, 2020

Variety	Treatment Scheme/ Witness	DA (%)	E (%)
Damian	I	1.11	83.3
	Control	6.65	
Matti	I	0.69	85.28
	Control	4.69	
Vangelis	I	1.40	78.85
	Control	6.62	
Tatry	I	1.18	80.20
	Control	5.96	
Damian	II	1.05	84.21
	Control	6.65	
Matti	II	0.58	87.63
	Control	4.69	
Vangelis	II	1.14	82.77
	Control	6.62	
Tatry	II	0.94	84.22
	Control	5.96	
Damian	III	0.58	91.27
	Control	6.65	
Matti	III	1.17	75.05
	Control	4.69	
Vangelis	III	1.17	82.32
	Control	6.62	
Tatry	III	0.75	87.41
	Control	5.96	

Efficacy of treatments in plant disease control is of particular importance in establishing schemes to combat them (Balasu et al., 2015; Mindru et al., 2018; Buzatu et al., 2018; Jalobă et al., 2019; Alexandru et al., 2019; Doncila 1995). The data in Table 3 show that the highest efficacy value of the treatment scheme was registered for the Damian variety under the conditions of treatment scheme III, at E = 91.27%. High efficacy values were obtained for the Matti variety in the case of

treatment scheme II (E = 87.63%) and Tetry in treatment scheme III at E = 87.41%. In the case of the Matti variety, the lowest efficacy value was registered in the case of treatment scheme III at E = 75.05%, followed by the Vangelis variety in scheme I (E = 78.85%), which can be attributed to its influence. For the Damian variety, an efficacy of over 83% was observed in all the treatment schemes tested. Research on the effectiveness of treatment schemes in controlling the sugar beet cercosporiosis has confirmed values of over 80% in decreasing the level of attack (Toth & Cristea, 2020). The application of

an integrated management has an effect on the attack of cercosporiosis on beets (Biancardi et al., 1999).

The influence of the treatment scheme on the production of roots was also followed and it was found that in all variants were obtained statistically guaranteed, very significantly positive production increases. The data in Table 4 show that the Damian variety had the highest average production of beet root, at 58.00 t/ha in treatment scheme 3, for which the degree of attack of micromycete and the effectiveness were the highest, as well.

Table 4. Sugar Beet Roots Production in the Locality Sânzieni, Covasna County, 2020

Experiment Scheme	Damian			Matti			Vangelis			Tatr		
	T / ha	% compared to MT	Significance	T / ha	% compared to MT	Significance	T / ha	% compared to MT	Significance	T / ha	% compared to MT	Significance
Experiment 1	56.50	131	***	56.20	139	***	49.80	132	***	53.50	134	***
Experiment 2	57.00	132	***	56.40	139	***	51.20	136	***	55.20	139	***
Experiment 3	58.30	135	***	54.60	135	***	48.70	130	***	51.80	130	***
Control (MT)	43.20	-	-	40.70	-	-	37.60	-	-	39.80	-	-
DL 5%	0.62			0.66			0.71			0.67		
DL 1%	0.82			0.87			0.94			0.89		
DL 0.5%	1.04			1.11			1.20			1.13		

## CONCLUSIONS

The application of treatments in different control schemes and the behavior of varieties continues to be important measures in controlling cercosporiosis at sugar beet. As a result of the application of treatments in vegetation, the degree of attack decreased considerably in all experimental variants compared to the witness. Under the conditions of the year 2020, for the Damian variety, in the experiment scheme 3 of the treatment, the highest value of efficacy was registered, E = 91.27%. In all treatment schemes production increases were obtained, the results being statistically assured.

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## THE QUALITY OF FRESH AND ENSILED BIOMASS OF *Brassica napus oleifera* AND PROSPECTS OF ITS USE

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### Abstract

The genus *Brassica*, family *Brassicaceae*, contains 38 species and many of them are known as important agricultural and horticultural crops, which provide edible roots, leaves, stems, buds, flowers and seeds, some are used as human food in many different forms, and some species are used as fodder and cover crops. We investigated some biological peculiarities and the quality of fresh and ensiled biomass of *Brassica napus* subsp. *oleifera*, which was cultivated on the experimental land in the National Botanical Garden (Institute), Chisinau. The green mass of *Brassica napus oleifera* was mowed in the flowering stage and some of its main biochemical parameters were assessed. Thus, crude protein (CP), ash (CA), acid detergent fibre (ADF), neutral detergent fibre (NDF), acid detergent lignin (ADL), total soluble sugars (TSS), digestible dry matter (DDM) and digestible organic matter (DOM) have been determined by near infrared spectroscopy (NIRS) technique using 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, and the sensorial and chemical characteristics of the prepared silage were determined in accordance with the standard for forage quality analysis SM 108. It has been determined that the fresh mass of *Brassica napus oleifera* contained 14.4% DM, 227 g/kg CP, 97 g/kg CA, 442 g/kg NDF, 285 g/kg ADF, 41 g/kg ADL, 244 g/kg cellulose, 157 g/kg hemicellulose, RFV=140, 13.07 MJ/kg DE, 10.73 MJ/kg ME and 6.75 MJ/kg NEL. These characteristics indicate a good quality of the natural feed for ruminants. The prepared silage contained dark-green leaves with yellow-green stems, the concentration of nutrients was: 231 g/kg CP, 152 g/kg CA, 467 g/kg NDF, 307 g/kg ADF, 34 g/kg ADL, 273 g/kg cellulose, 160 g/kg hemicellulose, RFV=129, 12.77 MJ/kg DE, 10.58 MJ/kg ME and 6.50 MJ/kg NEL. The biochemical methane potential of *Brassica napus oleifera* substrates reached 309-324 L/kg organic matter.

**Key words:** biochemical methane potential, *Brassica napus oleifera*, feed value, fresh mass, silage.

### INTRODUCTION

Adequate nutrition is essential for the reproduction and productivity of livestock. The forages should provide a stable and balanced diet for farm animals and meet the needs of ruminants to maintain their health and to improve the production of meat, milk and wool. Renewable energy sources coming from agricultural crops could play an important role in terms of energy supply and positive environmental effects. Biogas has become important as an alternative source of energy, because of its decentralized approach.

The family *Brassicaceae* Burnett (syn. *Cruciferae* Juss.) includes 372 genera and 4.060 accepted species. Most of them are herbaceous annuals, biennials and perennials, warm season shrubs and trees; some are used as agricultural crops and ornamental plants. The genus *Brassica* L. includes 38 species, are

native to Europe and temperate Asia and are especially common in the Mediterranean region, and the domestication of some of them dates back to antiquity. Many species are known as important agricultural and horticultural crops, which provide edible roots, leaves, stems, buds, flowers and seeds, some species are used as human food in many different forms, while others are also used as fodder, energy and cover crops. The most remarkable members include *Brassica napus* L., *Brassica oleracea* L., *Brassica juncea* (L.) Czern., *Brassica nigra* (L.) W.D.J. Koch., *Brassica rapa* L.

Rapeseed, *Brassica napus* L. subsp. *oleifera* DC., is an herbaceous annual, cultivated mainly for its oil-rich seeds, but also as forage and energy crop, with high potential as a honey plant, occurring commonly in areas with dry, temperate-continental climate. The stem is erect, branched, 70-130 cm tall, vigorous. The

leaves are bright green, covered with hairs, with well-defined veins, alternating, the basal ones are petiolate, lyrate, pinnatisect, but the middle and the upper ones - sessile, lanceolate or oblong-lanceolate, covered with a waxy layer. The inflorescence is a raceme with bright yellow hermaphroditic flowers, the flowering stage lasts 15-21 days, and the pollination is predominantly allogamous, entomophilous. The potential honey 242.2-324.8 kg/ha (Ion et al., 2012). The fruit is linear cylindrical silique, 3-5 cm long, with 10-30 round black-bluish to brown-bluish seeds; the weight of 1000 seeds is 3.5-6.5 g. The rapeseed has a tap root with few branches, which penetrates into the soil up to 300 cm deep, the main mass of roots is located at 25-45 cm depth. Winter cultivars of rapeseed utilize environmental resources more effectively and better protect the soil against erosion and nutrient leaching in autumn and winter, are predominant in Europe, their seed productivity, which is 20% to even 60% higher in comparison with spring varieties. Rapeseed oil is variously used in cooking, as an ingredient in soap and margarine, can be made into biodiesel, the residue after oil extraction is used for fodder. *Brassica* species have gained great importance as cover and fodder crops in cropping systems, due to their many environmental and agronomic benefits (Haramoto & Gallandt, 2004; Bell et al., 2020). The incorporation of rapeseed biomass can provide the soil with 7.7 t/ha of organic matter, 280 kg/ha N, 37 kg/ha P, 35.4 kg/ha Mg, 354.0 kg/ha K, 147.1 kg/ha Ca, 0.02 kg/ha Cu, 0.23 kg/ha Zn and 0.02 kg/ha Mn (Țiței & Mazăre, 2019). The cultivation of winter fodder rapeseed should be reconsidered, both in terms of fodder value and agroeconomic importance, in cropping systems, yields of 35-50 t/ha of green mass can be obtained. Winter rapeseed can be fed to animals very early (middle of April) and plays an important role in maintaining a continuity of fresh food supply for livestock (Dragomir, 2004). The aim of the current study was to evaluate some biological peculiarities, the quality of fresh and ensiled mass of winter rapeseed *Brassica napus* subsp. *oleifera* DC., as feed for ruminant animals, as well as substrate for the production of biomethane.

## MATERIALS AND METHODS

*Brassica napus* subsp. *oleifera* cv. 'Albatros', which was cultivated in the experimental plot of the National Botanical Garden (Institute) of Moldova, Chișinău, N 46°58'25.7" latitude and E 28°52'57.8", served as subject of study and the traditional crops alfalfa, *Medicago sativa*, and common sainfoin, *Onobrychis viciifolia*, were used as controls. The experimental design was a randomised complete block design with four replications, and the experimental plots measured 10 m<sup>2</sup>. *Brassica napus* was sown in late August at a depth of 2.0 cm in rows at a distance of 15 cm; the sowing density was 60 germinable seeds per m<sup>2</sup>. The plant growth, development and productivity were assessed according to methodical indications. The green mass was harvested in the flowering period. The green mass yield was measured by weighing. The dry matter content was detected by drying samples up to constant weight at 105°C. The leaf/stem ratio was determined by separating the leaves and flowers from the stem, weighing them separately and establishing the ratios for these quantities (leaves/stems). For ensiling, the green mass was shredded and compressed in well-sealed containers. After 45 days, the containers were opened, and the sensorial and chemical characteristics of prepared silages were determined in accordance with standard laboratory procedures and the Moldavian standard SM 108 for forage quality analysis. For chemical analysis, 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 of the main biochemical parameters were assessed: crude protein (CP), ash, acid detergent fibre (ADF), neutral detergent fibre (NDF) and acid detergent lignin (ADL), total soluble sugars (TSS), digestible dry matter (DDM), digestible organic matter (DOM) were determined by the 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 biogas potential (Yb) and the methane potential (Ym) were calculated according to the equations of Dandikas et al. (2014) based on the concentration of acid detergent lignin (ADL) and hemicellulose (HC):

$$Yb=727+0.25\text{ HC}-3.93\text{ ADL}$$

$$Ym=371+0.13\text{ HC}-2.00\text{ ADL}$$

## RESULTS AND DISCUSSIONS

While conducting the research on the biological peculiarities of *Brassica napus*, we observed that the emergence of the first seedlings occurred 4 days after sowing, and the abundant emergence – during the next 7-8 days. In late autumn, when the average temperatures dropped below 0°C, the rapeseed plants developed a rosette of 6-8 large leaves, and in the underground part - the main root, which was 8-10 mm thick and extended to a depth of over 25 cm. After a period of winter dormancy, the plants resumed growth in the first days of March. The first generative shoots emerged in late March - early April, and the flower buds - in the second half of April. The full flowering stage occurred in the first half of May.

Plant height, stem thickness and leaf/stem ratio have significant impact on the yield, but also affect the forage quality. Some results regarding the bio-morphological characteristics and the structure of the harvested aerial plant biomass are presented in Table 1.

At the harvest time, the height of *Brassica napus* plants was 125.1 cm, while the traditional leguminous forage crops reached 84.5-93.1 cm. *Brassica napus* had the largest weight of a single plant among the studied species. The forage yield of *Brassica napus* reached 58.2 t/ha green mass or 8.5 t/ha dry matter with 59.7% leaves and flowers, but the controls, *Medicago sativa* and *Onobrychis viciifolia* (first cut), respectively – 20.8-25.8 t/ha green mass or 5.6-6.1 t/ha dry matter with 46.5-50.5% leaves and flowers. The *Brassica napus* forage was richer in leaves, but poorer in dry matter, in comparison with the leguminous forage crops.

As a result of the research conducted in Hungary, Miko (2009) determined that the productivity of autumn rapeseed varied from 24.0 to 150.6 t/ha green mass, or 4.2-15.6 t/ha dry matter. Antanasovic (2012) mentioned that, in Serbia, autumn rapeseed could yield 77.1 t/ha green mass or 6.9 t/ha dry matter.

Table 1. Some agrobiological peculiarities and the structure of the green mass of the studied species

Plant species	Plant height, cm	Stem, g		Leaf, g		Yield, t/ha	
		fresh mass	dry matter	fresh mass	dry matter	fresh mass	dry matter
<i>Brassica napus</i>	125.1	123.74	11.81	120.97	17.52	58.2	8.5
<i>Medicago sativa</i> , first cut	84.5	5.38	1.38	4.92	1.41	20.8	5.6
<i>Onobrychis viciifolia</i> , first cut	93.1	12.50	2.86	10.10	2.49	25.8	6.1

Table 2. The biochemical composition and nutritive value of the harvested green mass of the studied species

Indices	<i>Brassica napus</i> fresh mass	<i>Brassica napus</i> silage	<i>Medicago sativa</i> fresh mass	<i>Onobrychis viciifolia</i> fresh mass
Crude protein, g/kg DM	227	231	172	166
Ash, g/kg DM	97	152	91	96
Acid detergent fibre, g/kg DM	285	307	347	309
Neutral detergent fibre, g/kg DM	442	467	510	447
Acid detergent lignin, g/kg DM	41	34	58	49
Total soluble sugars, g/kg DM	170	6	146	243
Cellulose, g/kg DM	244	273	289	260
Hemicellulose, g/kg DM	157	160	163	138
Digestible dry matter, g/kg DM	752	758	623	669
Digestible organic matter, g/kg DM	709	666	579	615
Relative feed value	140	129	118	142
Digestible energy, MJ/kg	13.07	12.77	12.20	12.73
Metabolizable energy, MJ/kg	10.73	10.58	10.03	10.46
Net energy for lactation, MJ/kg	6.75	6.50	6.04	6.48

Analyzing the results of the fresh mass quality of the *Brassica napus* (Table 2), we found that dry matter contained 227 g/kg CP, 97 g/kg ash, 285 g/kg ADF, 442 g/kg NDF, 41 g/kg ADL,

170 g/kg TSS, 244 g/kg Cel, 157 g/kg HC, with 75.2 % DMD, 70.9 % OMD, RFV=140, 13.07 MJ/kg DE, 10.73 MJ/kg ME, 6.75 MJ/kg NEI, but the leguminous forage crops - 166-

172 g/kg CP, 91-96 g/kg ash, 309-347 g/kg ADF, 447-510 g/kg NDF, 49-58 g/kg ADL, 146-243 g/kg TSS, 260-289 g/kg Cel, 138-163 g/kg HC, with 62.3-66.9% DMD, 57.9-61.5% OMD, RFV=118-142, 12.20-12.73 MJ/kg DE, 10.03-10.46 MJ/kg ME, 6.04-6.48 MJ/kg NEL. The natural fodder of *Brassica napus* was characterised by very high concentration of crude protein, optimal amounts of total soluble sugars and hemicellulose and lower content of acid detergent lignin, which had a positive effect on its digestibility and was able to meet the energy needs of farm animals.

Some authors mentioned various findings about the green mass quality of *Brassica napus*. According to Westwood & Mulcock (2012), the harvested whole plants contained 67.2% leaves and 31.3% stems, 14.3% DM, 10.8% CP, 2.9% lipids, 23.2% NDF, 20.3% ADF, 27.3% WSC, 9.1% ash. Çaçan & Kokten (2017) found that the chemical composition and feed value of the green mass of rapeseed was 15.5-18.1% protein, 40.7-44.1% ADF, 46.4-50.5% NDF, 54.5-57.2% DMD, RFV=101.8-114.9. Gül et al. (2019) reported that canola fresh mass contained 23.66% dry matter with 14.65% CP, 55.33% NDF, 44.80% ADF, 10.16% ADL, 4.94% WSC, 11.39% ash, 54.0% DMD, 60.79% OMD, RFV=90.77 and 8.93 MJ/kg metabolizable energy. Heuze et al. (2019) remarked that the average feed value of rapeseed fresh aerial part was: 12.1% dry matter, 15.8% protein, 3.8 % fats, 19.3% raw cellulose, 28.9% NDF, 23.2% ADF, 4.5% lignin, 14.7% ash, 21.7 g/kg calcium and 5.8 g/kg phosphorus, 79.2% digestible organic matter, 17.6 MJ/kg gross energy, 13.6 MJ/kg digestible energy and 10.6 MJ/kg metabolizable energy.

Forage preservation is a key element for productive and efficient ruminant livestock farms, which provides a uniform level of high quality feed for ruminants throughout the year. Silage is the main preserved green succulent roughage fed to domestic animals. When opening the glass vessels with *Brassica napus* silage, carbon dioxide, a by-product of fermentation, was eliminated in moderate amounts from the preserved mass. During the organoleptic assessment, it was found that silage had agreeable colour, dark-green leaves

and yellow-green stems, with specific aroma, somewhat like the smell of pickled cabbage; the consistency was retained in comparison with the initial fresh mass, without mould and mucus. It has been found that the concentration of nutrients and energy in silage was: 231 g/kg CP, 152 g/kg ash, 307 g/kg ADF, 467 g/kg NDF, 34 g/kg ADL, 6 g/kg TSS, 273 g/kg Cel, 160 g/kg HC, with 75.8% DMD, 66.6% OMD, RFV=129, 12.77 MJ/kg DE, 10.58 MJ/kg ME, 6.50 MJ/kg NEL. As compared with the initial fresh mass, the silage of *Brassica napus* had high concentration of ash, cellulose and very low content of total soluble sugars, which had a negative impact on digestibility, relative feed value and net energy for lactation.

Literature sources indicate considerable variation in the chemical composition and nutritional value of *Brassica* silages. According to Balakhial et al. (2008), the dry matter content and the biochemical composition of rapeseed silage was: 17.82% DM, pH = 4.78, 15.68% CP, 52.33% NDF, 32.33% ADF, 12.00% ash, 6.00% fats, 733.3 g/kg DMD, but rapeseed silages treated with different levels of urea and molasses - 18.79-20.21% DM, pH = 4.70-5.23, 15.64-16.67% CP, 47.66-50.66% NDF, 31.60-34.66% ADF, 11.67-12.67% ash, 3.33-4.33% fats, 586.6-760.0 g/kg DMD. Neely et al. (2009) reported that rapeseed silages contained 21.1% CP, 20.7% NDF, 19.9% ADF, 3.33-4.33% fats, 16.6% ash, RFV = 324. Sánchez et al. (2014) found that the dry matter content and the concentrations of nutrients and energy in *Brassica napus* wilted mass were: 308.6 g/kg DM, 277.7 g/kg CP, 302.2 g/kg NDF, 264.9 g/kg ADF, 225.7 g/kg ash, 202.0 g/kg NFC, 535.6 g/kg TDN, 1.33 Mcal/kg NEL, 2.16 Mcal/kg ME, but in the prepared silage: pH = 4.2, 346 g/kg DM, 126.8 g/kg lactic acid, 110.4 g/kg acetic acid, 7.4 g/kg propionic acid, 259 g/kg CP, 258 g/kg NDF, 235 g/kg ADF, 205 g/kg ash, 315 g/kg NFC, 598 g/kg TDN, 1.48 Mcal/kg NEL, 2.38 Mcal/kg ME. Herrmann et al. (2016) studied the biochemical composition of silages made of various crops in Germany and remarked that *Brassica napus* silage contained 213-364 g/kg dry matter with 89.7-92.0% organic matter, pH 3.8-4.5, 3.7-10.6% lactic acid, 1.1-2.8% acetic acid, 0-0.3% butyric acid, 8.9-11.3% CP, 3.4-17.2% fat, 41.2-53.4%

NDF, 34.6-51.2% ADF and 5.7-11.4% ADL. Gül et al. (2019) mentioned that rapeseed silage contained 21.79% dry matter with pH=4.63, 3.6 g/kg lactic acid, 14.31% CP, 54.32%NDF, 41.20% ADF, 19.37% ADL, 1.4% WSC, 13.85% ash, 56.80% DMD, 61.45% OMD, RFV = 97.24 and 7.94 MJ/kg metabolizable

energy. Heuze et al. (2019) revealed that rapeseed silage contained 17.2% dry matter, 16.4% protein, 3.8% fats, 18.7% raw cellulose, 29.9% NDF, 23.8% ADF, 5.4% lignin, 19.0% ash, 9.8 g/kg calcium and 0.9 g/kg phosphorus and 17.6 MJ/kg gross energy.

Table 3. The biochemical biogas and biomethane production potential of the investigated substrates

Indices	<i>Brassica napus</i> fresh mass	<i>Brassica napus</i> green mass	<i>Medicago sativa</i> fresh mass	<i>Onobrychis viciifolia</i> fresh mass
Minerals, g/kg DM	97	152	91	96
Nitrogen, g/kg DM	36.3	37.0	27.5	26.6
Carbon, g/kg DM	501.7	471.1	505.5	502.2
Ratio carbon/nitrogen	13.8	12.7	18.4	18.9
Cellulose, g/kg DM	244	273	289	260
Hemicellulose, g/kg DM	157	160	163	138
Acid detergent lignin, g/kg DM	41	34	58	49
Bio gas potential, L/kg VS	605	633	540	569
Biomethane potential, L/kg VS	309	324	276	291

Plant biomass may be used for biogas production directly after harvest and as ensiled substrates. Anaerobic decomposition will produce methane, carbon dioxide, some hydrogen and a final product that can be used as a fertilizer. 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, due to low pH, poor buffering capacity and high concentrations of ammonia in the substrate. Dobre et al. (2014), mentioned that the optimal C/N ratio is expected to be in the range 15-25, when the anaerobic digestion process is carried out in a single stage, and for the situation when the process develops in two steps, the optimal C/N ratio will range: for step I: 10-45; for step II: 20-30. The results regarding the quality of the *Brassica napus* substrates and the potential for obtaining biomethane are shown in Table 3. The nitrogen content in the studied *Brassica napus* substrates ranged from 3.63% to 3.7%, the estimated content of carbon - from 47.11% to 50.17%, the C/N ratio varied from 12.7 to 13.8, but in the green mass substrates of leguminous crops, there was 2.66-2.75% nitrogen, 50.22- 50.55% carbon and C/N = 18.4-18.9. Essential differences were observed between the lignin contents. The *Brassica napus* substrates contained acceptable amounts of hemicellulose and low amounts of lignin; the

biochemical methane potential reached 309-324 l/kg VS. The best methane potential was achieved in *Brassica napus* silage substrate - 324 l/kg VS, the lowest - in the fresh mass substrate of *Medicago sativa*.

According to Zubr (1986), the methane potential of fresh rapeseed substrate was 334 l/kg, but - of rapeseed silage substrate – 330 l/kg. Cleemput (2011) found that the methane values of hybridized winter rapeseed substrates ranged from 275.38 to 396.71 l/kg ODM. Murphy et al. (2011) reported that oilseed rape produced 2.5-7.8 t/ha dry matter and the measured methane yield was 240-340 m<sup>3</sup>/t VS. Herrmann et al. (2016) mentioned that, in rapeseed silage substrates, the C/N ratio was 24-31, the methane content - 57.3-62.8 % of the produced biogas, and the biochemical methane potential was 244.2-276.3 l/kg ODM.

## CONCLUSIONS

Under the climatic conditions of the Republic of Moldova, autumn rapeseed, *Brassica napus oleifera*, grows and develops optimally. In the flowering stage, early May, the forage yield can reach about 58.2 t/ha green mass or 8.5 t/ha dry matter.

The forage dry matter contains 227 g/kg CP, 97 g/kg ash, 285 g/kg ADF, 442 g/kg NDF, 41 g/kg ADL, 170 g/kg TSS, 244 g/kg Cel, 157 g/kg HC, with 75.2% DMD, 70.9% OMD,

RFV=140, 13.07 MJ/kg DE, 10.73 MJ/kg ME, 6.75 MJ/kg NEL.

*Brassica napus* silage contains 231 g/kg CP, 152 g/kg ash, 307 g/kg ADF, 467 g/kg NDF, 34 g/kg ADL, 6 g/kg TSS, 273 g/kg Cel, 160 g/kg HC, with 75.8% DMD, 66.6% OMD, RFV=129, 12.77 MJ/kg DE, 10.58 MJ/kg ME, 6.50 MJ/kg NEL.

The biochemical methane potential of fresh mass and silage substrates of *Brassica napus oleifera* reaches 309-324 L/kg organic matter.

*Brassica napus oleifera* can be used as alternative feed for ruminants and as substrate for biomethane production.

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## PHYTOSANITARY CONDITION OF RAPESEED IN THE REGION OF KAVARNA

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### Abstract

*Rapeseed is grown because of the seeds which are rich in fat (30-49%). This makes it the most important plant rich in oil and protein in temperate climate zone. Thanks to this, in the last few decades rapeseed has taken the honorable third place in the world production of vegetable oils. The aim of the present study is to establish the phytosanitary condition of the cultivated rapeseed in the area of the Kavarna town. The present study includes four cultivars of rapeseed grown in the area. As a result of the study it was found that the studied cultivars are characterized by high winter hardiness - 90.5% and good productivity 359.8 kg/da. The rapeseed oil reaches an average content of 47.8%. The low content of glucosinolates in the seeds of rapeseed cultivars 13.3  $\mu\text{mol/g}$  allows quality oil production. The average erucic acid content is 1.31%. Economically important fungal diseases in the autumn for the area of Kavarna town are *Phoma* (*Leptosphaeria maculans*), *Alternaria* (*Alternaria brassicae*) and Powdery mildew.*

**Key words:** rapeseed, productivity, seeds, cultivars, oil.

### INTRODUCTION

Rapeseed (*Brassica napus L.*) belongs to the family *Brassicaceae*, genus *Brassica*. It is grown for seeds that are rich in fat (30-49%). This makes it the most important plant rich in oil and protein in the temperate zone. Thanks to this, in the last few decades rapeseed has taken an honorable third place in the world's vegetable oils. The waste products, - in this production – cake and meal - contain about 33 % protein and 10 % fat and are valuable feed for farm animals. Meal from eruciless cultivars is close to soybean meal in terms of forage value.

In recent years, rapeseed oil is no longer used only for food, but also as fuel. The reduction of the content of glucosinolates in rapeseed from 80  $\mu\text{mol/g}$ . to the limit below 10  $\mu\text{mol/g}$ , allows the use of waste products (cake, meal) for feed. These cultivars are marked with 00 (two - zero, canola) with a very low content of erucic acid.

The local production is facing reconstruction. Increased demand for alternative energy sources has created a stable market. Europe's need for rapeseed is growing dynamically. Along with the supply of renewed cultivars and hybrids, the production technology is becoming more and more intensive. In recent

years, between 1.3 and 1.8 million decares of rapeseed are harvested annually in Bulgaria (Raykov et al., 2009).

The growth cycle of winter oilseed rape takes place in autumn and spring and goes through the following few characteristic phases. The first two phases of development take place in autumn. Spring growth is evident in the months of February - March, when the air temperature is permanent + 5°C or more (Kyuchukova, 1983).

Winter oilseed rape has a good regenerative ability and can relatively well restore the loss of leaf mass from winter. When growing winter oilseed rape, there are certain climatic requirements and requirements for the type and condition of the soil (Tonev, 2000; Tonev, 2006). Climatic requirements: these requirements include the needs of the crop for heat, light and moisture. Rapeseed requires a temperate climate. Rapeseed frost hardiness sometimes helps in the successful weed control (some of the main weeds accompanying this crop do not tolerate such low temperatures) (Fetvadjeva et al., 1988).

Rapeseed develops a large mass and needs a lot of water, especially during budding and flowering, as well as the growth of pods. The seeds germinate when absorbing 80-90% of water from their mass, and during flowering

this crop requires high atmospheric humidity, nearly 80 %. Average annual rainfall of 550-700 mm is favorable for achieving optimal yields. In our country rapeseed is attacked by many diseases, mainly fungal. In the past, rapeseed was considered a crop that did not need fungicidal protection and almost no attention was paid to rapeseed diseases. Currently, in rapeseed crops, due attention should be paid to fungal diseases, especially Foma and White rot (Nankov, et al., 1994; Nakov et al., 1999; Shindrova, 2015).

Some rapeseed pathogens are polyphagous, respectively, they can be transmitted to it from other crops (*Sclerotinia sclerotiorum*, *Botrytis cinerea*, soil pathogens), but most of the rapeseed pathogens are specific to plants of the cruciferous family - *Brassicaceae* (BobeV, 2009b).

The purpose of this study is to establish the phytosanitary condition of cultivated rapeseed in the region of Kavarna.

## MATERIALS AND METHODS

In the last few years, in the region of Kavarna, cultivars of Rapul (a German company with a representative in Bulgaria) have been sown. Our study included four cultivars grown in the area, namely Sherpa, Dalton, Edimax and Shrek. The company's portfolio presented cultivars with the following selection indicators. Sherpa cultivar is characterized by very good frost hardiness and rapid recovery of spring growth. This is a universal hybrid, suitable for late sowing, with the application of minimum tillage technologies, as well as for growing in less favorable conditions. This is due to its adaptability and the powerful strong root system of the hybrid, which reaches a depth of 2 m. as a total length of the roots, and the lateral branches and active root pappus, absorb nutrients and water to the maximum efficient way. The Dalton cultivar is a representative of a new generation of Rapul hybrids. It is selected especially for the conditions of Southern Europe and combines many advantages necessary for the successful production of rapeseed in Bulgaria. One of them is disease resistance in particular to Foma, thanks to the gene Rlm 7, thus preventing the negative effects on the yield due to infection

with the fungus *Foma Lingam*. Another extremely important advantage of the cultivar is the strong growth in the autumn, thanks to which the hybrid is extremely suitable for late sowing, and even then it guarantees optimal development for overwintering.

Edimax cultivar is suitable for all soil types and different production systems – either extensive or intensive. It has an extremely fast initial start in autumn, as a result of which the hybrid is suitable for medium late and late sowing dates. This cultivar is selected for cultivation in continental conditions. Its excellent frost hardiness and drought and stress tolerance makes it suitable for growing in all regions of the country.

Cultivar Shrek is very suitable for later sowing dates. Thanks to the quick start, we observe a very good autumn development, without the risk of stem outgrowth. Its genetics makes it extremely suitable for the “golden” autumn conditions of Bulgaria. The pods are significantly longer and over 20% heavier than those of other hybrids. This is of great importance in regions with severe droughts or in cases of late spring frosts, because the seeds in the other pods reach a higher absolute mass and thus we get a high yield, regardless of adverse conditions.

Kavarna municipality has flat to - hilly terrain, with slightly vertical and horizontal dissection, the predominant slopes are from 3 to 5 degrees. The municipality covers the eastern part of Danube plain - the southern parts of the Dobrudzha plateau, and parts of the Black Sea coast – coasts and shelf. The territory's coast, has clearly expressed old and modern abrasion and accumulation processes. From Cape Shabla to Cape Kaliakra, the abrasion has formed numerous caves, underwater and surface cliffs. Kavarna municipality belongs to the area of the temperate – continental climate, which is formed under the direct influence of the Black Sea. The region is relatively cold for its latitude with average temperatures of + 0.8 °C in January and 22.3°C in July. The annual temperature amplitude is 21.7-21.9°C. The average annual air temperature is +11.8°C. The region belongs to the driest regions of Bulgaria. The total average annual rainfall is in the range of 411-480 mm. The maximum precipitation amounts for the region of Kavarna municipality

are marked in autumn, the minimum in spring and summer. (www. government.bg.net, www. Meteoshum. bg.net).

The area is completely occupied by chernozem soils with their varieties - carbonaceous, residual - forest and typical. Chernozem soils have strong humus horizon, fertility, with a slightly alkaline to neutral soil reaction.

In our study, the examined cultivars were located in the land of Kavarna town. The area of the individual cultivars was within 150-180 da. The crops are grown using the traditional technology. Sowing was carried out on 25 August. The sown hybrids are grown for the first time by the company. The inspection of the cultivars was carried out three times, in the autumn after the germination of the crop (October - 15), in the month of April (April 20) and along with the ripening of the crops (June 20). To determine the frost hardiness of the cultivars we used the meters, which were placed by the agricultural company. Reporting took place in the autumn, when we determined the disease attack, and also in the spring. Reporting to disease attack was done through an individual assessment of 100-150 plants. We analyzed symptomatic picture of randomly selected plants. A five-point scale was used to score from zero to 100%.

- 0 - no disease
- + - 0% to 25% - weak disease attack
- ++ - 26% to 50% - average disease attack
- +++ - 51% to 75% - severe disease attack
- ++++ - 76% to 100% - very strong disease attack.

We made visual diagnosis based on the symptoms of the disease. For specific studies, visual diagnostics was supported by the use of specialized guides and reference books (BobeV, 2002; Bobev, 2009a; Stancheva, 2002; Stancheva, 2006).

The average yields were reported after the harvest, the data was kindly provided by the producer. The technological markers of the raw material quality are checked by the companies that bought the rapeseed. The aim of the present study is to establish the phytosanitary condition of the cultivated rapeseed in the area of Kavarna town.

## RESULTS AND DISCUSSIONS

Rapeseed is a risky crop for our country. It is mainly due to the fact that the critical temperature at which rapeseed freezes is minus 17 degrees. Each year the frost leads to lighter losses in different regions. Large losses are typical for long winters with severe cold and temperature fluctuations, especially after a warm autumn. Figure 1 presents the results of the examination of the cultivars for their frost hardiness. In the spring, while reporting the diseases, we counted the overwintering plants and from the difference in the autumn we calculated the percentage of frost hardiness. The results of our study show that the highest percentage (93.6%) overwintered plants is in Edimax cultivar, which was confirmed by the portfolio of the cultivar. The other cultivars show values close to the average for the group, which is 90.5%. This data gives us reasons to conclude that the cultivated cultivars of rapeseed in Kavarna region show good frost hardiness.

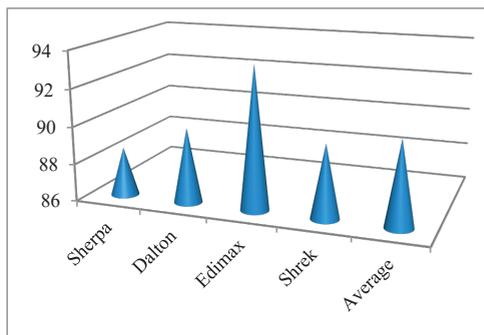


Figure 1. Frost hardiness of rapeseed cultivars in %

To determine the productive potential of rapeseed cultivars we take into account the obtained average yields in kg/da, grain (Figure 2). For the studied period the average yield of seeds from the studied cultivars reached 359.8 kg/da. The highest average seed yield per decare was obtained by the Sherpa cultivar - 388.1 kg/da. The superiority over the average for the group is 7.7%. Edimax cultivar (361 kg/da) has a relatively lower yield.

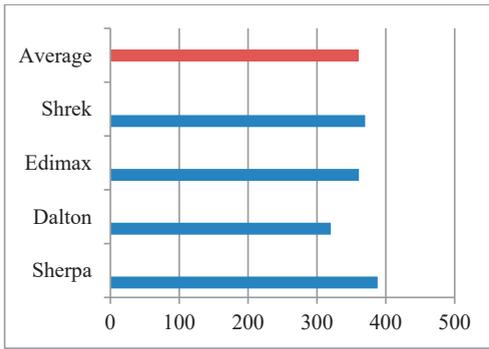


Figure 2. Average rapeseed yields in kg/da

The technological qualities of modern cultivars are evaluated by the content of rapeseed oil in the seeds. Figure 3 presents graphically the studied cultivars data on the oil content in percentage.

For the studied hybrids, the average oil content in the seeds is 47.9%. The highest oil content in the seeds (49.0%) was found in Edimax cultivar where the excess is 10.1 % over the average for the group.

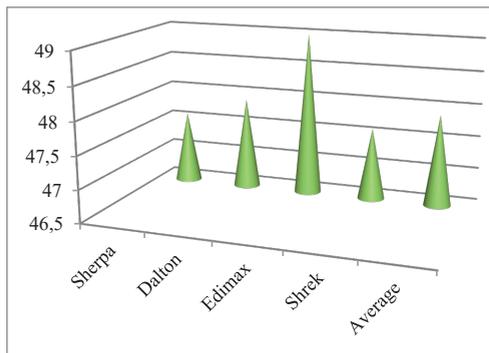


Figure 3. Oil content in rapeseed in %

With the progress of rapeseed selection and the establishment of new high standards in the quality of rapeseed, which is marked with “00”, the content of erucic acid in the oil must be below 2 % and 25  $\mu\text{mol/g}$  glucosinolates in the grain. It is believed that the lower the content of glucosinolates (below 15-25  $\mu\text{mol/g}$ ) in rapeseed, the better the quality of the oil and meal obtained. Our study of these indicators is presented in figures 4 and 5. The content of glucosinolates in rapeseed for the four cultivars is 13.1  $\mu\text{mol/g}$ . The lowest content of glucosinolates in the seed was reported in

cultivar Edimax 11.8  $\mu\text{mol/g}$ . In the case of Sherpa hybrid, this content is 13.8  $\mu\text{mol/g}$ . Approximately the same values were found for Shrek hybrids - 13.6  $\mu\text{mol/g}$  (Figure 4).

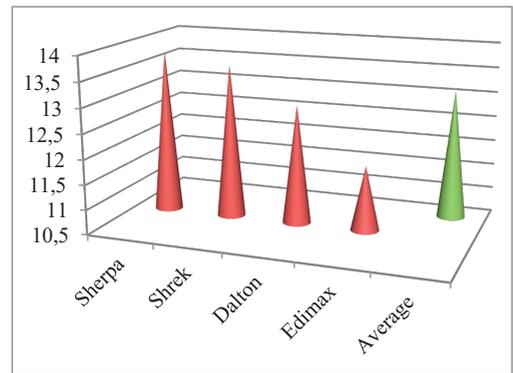


Figure 4. Content of glucosinolates in rapeseed in  $\mu\text{mol/g}$

Figure 5 presents the results of the content of erucic acid in the oil of the studied rapeseed cultivars. It is known from the literature that rapeseed oil contains erucic acid, which is toxic to humans and animals.

Modern selection using the method of genetic engineering creates cultivars, including genetically modified ones, in which the content of erucic acid is about and below 2%.

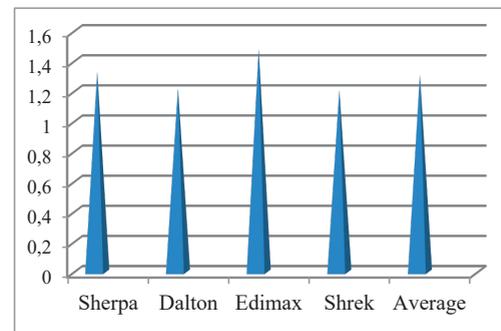


Figure 5. Erucic acid content of rapeseed, %

The oil from these cultivars is almost harmless for humans and is mainly used for margarine production. We could obtain results through chemical analysis for the studied rapeseed hybrids and they show that the highest erucic acid content of 1.48% is found in Edimax cultivar. Dalton (1.22%) and Shrek hybrids (1.21%) have approximately similar content.

The average erucic acid content for the group was 1.31%.

The obtained results for the productive and quality indicators of the studied cultivars show that they are characterized by good productivity as the average seed yield is 359.8 kg/da. The low content of glucosinolates in rapeseed - 13.1 μmol/g, allows the production of quality oil. The highest content of erucic acid was found in Edimax cultivar - 1.48%. The average erucic acid content is 1.31%. In our country, rapeseed is attacked by a number of diseases - some of them with economic influence are: sclerotic rot, dry stem rot (phomosis), black leaf spots (black spots or alternaria), mold, light leaf spots, powdery mildew and others.

We tried to make a tabular outline of the main diseases of rapeseed crops in late autumn, spring and the end of the growing season.

Table 1 shows the estimates of the rapeseed disease attacks on the studied rapeseed cultivars in autumn. Timely sowing and good pre-sowing soil preparation allowed the rapeseed crop to develop normally, which is a prerequisite for good overwintering of the crop. The data show that a relatively higher attack was reported by the diseases Foma, Alternaria and Powdery mildew. Analyzing the results, we found that the Dalton cultivar showed very high resistance to Foma and Shrek has resistance to Powdery mildew. In the autumn there was no attack of Sclerotinia and very weak attack of fungal diseases Gray rot and White rust.

Table 1. Rapeseed disease attacks in autumn

Diseases	Sherpa	Dalton	Edimax	Shrek
White rot ( <i>Sclerotinia sclerotiorum</i> )	0	0	0	0
Foma ( <i>Phoma lingam</i> )	+	0	++	++
Black spots or Alternaria ( <i>Alternaria brassicae</i> )	++	++	++	++
Mold ( <i>Peronospora parasitica</i> )	0	0	+	+
Gray rot ( <i>Botryotinia fuckeliana</i> )	0	0	0	+
White rust ( <i>Albugo candida</i> )	0	+	0	0
Powdery mildew ( <i>Erysiphe cruciferarum</i> )	+	++	+	0
Cilindrosporiosis ( <i>Pyrenopeziza brassicae</i> )	0	0	+	+

In the spring we reported the attack of fungal diseases on the studied hybrids (Table 2).

From the presented data it is evident that in the spring the cultivars were attacked mainly by

the diseases foma (*Phoma lingam*), gray rot (*Botryotinia fuckeliana*) and cilindrosporiosis (*Pyrenopeziza brassicae*).

Table 2. Rapeseed disease attacks in April

Diseases	Sherpa	Dalton	Edimax	Shrek
White rot ( <i>Sclerotinia sclerotiorum</i> )	+	0	0	0
Foma ( <i>Phoma lingam</i> )	+	0	+	++
Alternaria, black spots ( <i>Alternaria brassicae</i> )	0	+	0	+
Mold ( <i>Peronospora parasitica</i> )	0	0	0	+
Gray rot ( <i>Botryotinia fuckeliana</i> )	++	+	++	+
White rust ( <i>Albugo candida</i> )	0	+	0	0
Powdery mildew ( <i>Erysiphe cruciferarum</i> )	+	+	+	0
Cilindrosporiosis ( <i>Pyrenopeziza brassicae</i> )	+	++	+	+

The strongest attack was made by the fungal disease foma. The second place in terms of damage is gray rot. This disease was found at the base of the young stems and parts of the leaves where there were rotten areas as well as gray sticker. Dalton cultivar also showed complete resistance to Foma in the spring.

The last disease report was taken at the end of the growing season. Reporting coincided with the end of flowering with over 60% of the pods already formed.

Table 3. Rapeseed disease attacks in June

Diseases	Sherpa	Dalton	Edimax	Shrek
White rot ( <i>Sclerotinia sclerotiorum</i> )	++	++	+	++
Foma ( <i>Phoma lingam</i> )	+	0	+	+
Black spots or Alternaria ( <i>Alternaria brassicae</i> )	++	++	++	++
Mold ( <i>Peronospora parasitica</i> )	++	++	++	+
Gray rot ( <i>Botryotinia fuckeliana</i> )	0	0	0	0
White rust ( <i>Albugo candida</i> )	0	0	0	0
Powdery mildew ( <i>Erysiphe cruciferarum</i> )	+	0	+	0
Cilindrosporiosis ( <i>Pyrenopeziza brassicae</i> )	0	0	0	0

White rot, Mold and *Alternaria* showed the highest degree of development. Estimates of *Alternaria* attacks show that all the cultivars were almost equally affected by the disease. The highest harmful effect of the disease was observed in young pods, which were deformed, wrinkled, cracked, so that it led to immature seeds fall. The studied cultivars showed very good resistance to fungal diseases such as Gray rot, White rust, and *Cilindrosporiosis* at the end of the growing season.

## CONCLUSIONS

The studied cultivars are characterized by high frost hardiness - 90.5% regarding the questioned year and the region's climatic conditions.

The studied cultivars have relatively good productivity, on average 359.8 kg/da. Sherpa cultivar is characterized by the highest yield (388 kg/da) compared to the others.

The rapeseed oil content reached an average of 47.9% for the group. The low content of glucosinolates in the seeds of rapeseed cultivars - 13.3 µmol/g, allows the production of quality oil.

The highest content of erucic acid was found cultivar Edimax cultivar - 1.48%. The average erucic acid content for the group was 1.31%.

Economically important fungal diseases in the autumn for the area Kavarna town are Foma, *Alternaria* and Powdery mildew. At the end of the growing season, the highest level of development is found for diseases, White rot, *Alternaria* and Mold.

It was found that the studied cultivars showed very good resistance to fungal diseases, such as Gray rot, White rust and *Cilindrosporiosis* at the end of the growing season.

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## TRAP CROPPING: A USEFUL APPROACH IN FARMING SYSTEMS

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### Abstract

*Trap crops are considered a useful strategy for insect pest control and offer several benefits in a pest management system. The principle of trap cropping relies on pest preference for certain plant species, cultivars or a certain stage of crop development. Farmers are motivated to utilize trap cropping because of the various difficulties in coping with the pest situation in other ways. Trap cropping, the use of alternative host plants to attract, intercept and/or retain targeted insect pests for the purpose of reducing damage to a main crop, is one such strategy. This paper aims at presenting various crops suitable for an insect trap, which may be used in farms, as for the organic ones a sweep net shall be used to collect insect pests instead of pesticides. When trap crops successfully attract pest populations, treatments are localized only in this area, thus limiting the damage to the main crops. The two main techniques used in choosing a trap crop will be considered. We will look at the different types of trap crop locations in the study area. The so-called 'push-pull' strategy shows very good results in Kenya. Whether using the same or different species, it is essential that the trap crop is more attractive than the main crop. Examples will be given of perimeter trap cropping, row and strip trap cropping, as well as their effectiveness in fiber, trench, cereals and vegetable crops in the USA, Thailand, Kenya, New Zealand and others. The advantages of trap crops in reduction of usage of insecticides are highlighted: reduction of pest incidence to manageable levels; conservation of indigenous natural enemies by providing shelter to be useful in the agroecosystem; growing harvested crops trap. The shortcomings mentioned by the farmers are reported in order to clarify their use.*

**Key words:** *insect trap, push-pull' strategy, Trap crops.*

### INTRODUCTION

Interest in trap cropping, a traditional tool of pest management, has increased considerably in recent years (Shelton & Badenes-Pérez, 2006). Trap crops are considered a useful strategy for insect pest control (Sharma et al., 2019) and offer several benefits in a pest management system (Wszelaki & Broughton, 2013). For agricultural pest management, trap cropping potentially reduces crop damage, inexpensively and simultaneously reduces conventional pesticide applications (Cavanagh et al., 2009; Lu et al., 2009). Farmers are motivated to utilize trap cropping because of the various difficulties in coping with the pest situation in other ways (Hokkanen, 1991).

This paper aims at presenting various crops suitable for an insect trap, which may be used in farms, as for the organic ones a sweep net shall be used to collect insect pests instead of pesticides.

Sarkar et al. (2018) points out that according to Hokkanen (1991) since the 1930s there have been numerous reported cases of successful

trap cropping for managing various insect pests, ultimately resulting in a substantial reduction in the use of pesticides in developing countries. Sharma et al. (2019) presents a large number of authors who cite successful examples of the use of trap crops since the late 1900s for insect pest management on cotton, soybeans, potato, rape, beans, rice, sorghum, vegetable crops and others. Tillman & Cottrell (2012) reports that according to Hokkanen (1991) the cultivation of a trap crop is a pest management strategy that uses an attractive plant species that retains pests and reduces the likelihood of them entering the main crop.

### RESULTS AND DISCUSSIONS

A trap crop, also known as a sacrificial crop, that attracts pests and keeps them away from the main crop, which is why these valuable organic techniques are regularly used in organic farms (Dore, 2010).

Organic producers can stagger upon trap crops and use these tactics to mechanically remove insects (Majumdar et al., 2012). Sarkar et al.

(2018) mention that according to Zehnder et al. (2007) in organic crop production pest management relies primarily on habitat manipulation through farm scaping and other biological control practices.

There are two primary techniques used in the selection of trap cropping: selection of a more preferred plant species or cultivar grown at the same time as the main crop; planting of the same species and cultivar as the main crop timed to be at the most preferred stage of development before the main crop (Wszelaki & Broughton, 2013). The principle of trap cropping relies on pest preference for certain plant species, cultivars or a certain stage of crop development (Hokkanen, 1991; Fenoglio et al., 2017). In order for a crop to function successfully as a trap crop, there are two main processes of attracting the pest to the trap plant and retention of pests that arrive (Holden et al., 2012). Because insect invasions happen at specific times of the year, trap crops need to be sown in time to well established by the time pests arrive (Dore, 2010). Trap crops are generally attractive to pests during their reproductive stages; hence, trap crops are planted earlier than the main crops (Majumdar et al., 2012; Khan, 2015). Majumdar et al., (2012) cite Shelton & Badenes-Pérez (2006) and Wszelaki & Broughton (2010), who point out that trap crops are grown to lure pests away from the main crop by providing an alternative place for insects to feed and lay eggs. The supply of attractive host plants at the critical time in the pest's and/or the crop's phenology leads to the concentration of pests at the desired site, the trap crop (Hokkanen, 1991). Whether the same or different species are used, it is essential that the trap crop is more attractive than the main crop (Wszelaki & Broughton, 2013). Techniques of manipulation range from establishing an early or a late trap crop of the same cultivar as the main crop to planting a completely different plant species (Hokkanen, 1991). The amount of trap crops depends on the insect, which must be limited, as Parker and Snyder (2014) recommends that the trap crop is 10% of the crop used. According to Dore (2010) farmers usually set aside about 20% of the main crop area for the trap crop, which also may lessen dispersal out of the trap crop (Michaud et al., 2007). Sarkar et al. (2018)

report the indication of Holden et al. (2012) that the trap crop should cover not more than 2-10% of the total crop area.

When trap crops successfully attract pest populations, the treatments are localized only in this area and the pests do not reach the main crop, thus limiting the damage to the crop (Wszelaki & Broughton, 2013). There are a number of publications that state that applying a pesticide directly to the trap crop is the most common method of controlling pests. This has worked in systems ranging from Lepidopteran pests of *Brassica oleracea* to *Acalymma vittata* and *Anasa tristis* on cucurbit crops (Dogramaci et al., 2004; Holden et al., 2012). Treating trap crops with insecticides at peak activity is an effective strategy to reduce pest populations (Majumdar et al., 2012). Plants produce chemicals, or volatiles, that attract insects for pollination and repel pest insects. Different species and cultivars produce varying degrees of unique volatiles substances that repel insect pests more strongly than others and this makes them suitable for trap crops (Wszelaki & Broughton, 2013). The different types of used trap crop 'complement' each other by providing a mixture of chemical profiles (different primary glucosinolates and concentrations of them); different physical structures (glossy leaves vs. hairy leaves) and different phenologies (rape matures later in the season) (Parker & Snyder, 2014).

Trap cropping, the use of alternative host plants to attract, intercept and/or retain targeted insect pests for the purpose of reducing damage to a main crop, is one such strategy (Holden et al., 2012). Since there is no single trap crop that can attract all pest species, the selection of trap crops depends on the target insect pest, while design or layout depends on the available field space, insect migration, and grower's preference (Majumdar et al., 2012). The layout of the trap crop should be according to the specific pest, as for some insects it is sufficient to plant the trap crop around the border, and for insects that are harder to stop, a different arrangement is used (Dore, 2010). In a presentation Majumdar (2011) points out that when the method of planting - border crop is used, a successful control of *Heliothis* sp. carried out through a castor trap crop, in the main cotton crop. An Internet source (PAN

Germany, 2005) lists trap crops that are used in the cultivation of cotton, with a suitable border crop okra as a trap crop against *Flower cotton weevil* (Hasse, 1987). When growing corn, the ICIPE (2003) recommends growing a border crop of Napier grass and Sudan grass to control stemborer. In a publication Sharma et al. (2019) indicates that the earliest successful examples of trap crops for insect pests management include management of boll weevil and *Helicoverpa armigera* on cotton; and the European corn borer and *Helicoverpa zea* (Boddie) on maize. As specified in all these examples, early planting of the same crop was used as trap crops. Shelton & Badenes-Pérez (2006) cites authors (Hunt & Whitfield, 1996; Hoy et al., 2000) who point out that early-planted potatoes were used as a border trap crop for the Colorado potato beetle moving from overwintering sites to crops attacked by it and concentrating in the outer rows, where it could be treated with insecticides, cultural practices, or even propane flammers. Sharma, (2013) quotes Thiery & Visser (1987), according to who the Colorado potato beetles are attracted to volatiles from potato, but are repelled or not attracted by mixtures of potato and tomato. Majumdar (2011) and Dore (2010) report that it is recommended against slugs in vegetables to form a border crop of chervil. Nettle attracts aphids and Nasturtiums are also very attractive to aphids (blackfly, greenfly, whitefly), while radish attracts *Flea beetle* and Root fly away from cabbages (Dore, 2010). For effective control of thrips it is recommended to use basil and marigold as a border crop in the main crop of garlic (Majumdar, 2011), and in the crop of pepper successfully results are achieved by the formation of a border crop with corn (Vlahova, 2013). Parker et al. (2013) presents an example of pacific gold mustard as a crop trap, which is flanked on both sides by the main crop of broccoli. The main mechanism of operation of the trap crop shows that the trap crop is more attractive than the main crop. The mustard crop is often used to attract pests away from broccoli. Sharma et al., (2019) cite Criddle (1922), according to who Rye grass was used as the trap crop in ditches and headlands of wheat fields to provide a place for laying eggs of *C. cinctus* and were later destroyed crop. The same study reports

that brome grass can also be a superior trap crop because the survival rate of *C. cinctus* larvae is considerably lower on this plant and also the parasitism rates are greater on this grass. Another crop - fall rye, has also been found to be an effective trap crop for *C. cinctus* management.

Parker and Snyder (2014) presents two specific ways of arranging a perimeter trap crop-as a boundary surrounding the main crop and a strip trap crop on one side of the crop. Boucher et al. (2003) points out that perimeter trap crops can be defined as a planted crop around the border of the main crop. Shelton & Badenes-Pérez (2006) specifies that more attractive plants are used for insect control. Boucher & Durgy (2004) cite many authors who determine that perimeter trap cropping has led to a dramatic increase in trap crop efficacy over the past decade on a variety of pests and crops. But they clarify that perimeter trap cropping does not work on every pest or for every crop. The authors report that perimeter trap cropping functions by intercepting pest migration, concentrating the pest population in the border area, where they can be controlled, thus preserving natural enemies in the main crop. Boucher & Durgy (2004) recommend applying foliar insecticides to the perimeter crop as soon as beetles are found or their feeding begins on the trap crop, without waiting for a threshold level to be exceeded. A perimeter trap crop system incorporating sorghum (NK300) and Peredovik sunflower in Clanton, Alabama provides significant reduction in pesticide usage on tomatoes as a main crop. Insecticide treatment of sorghum at peak leafhoppered bug activity provides 78 to 100% control of the pest without the need to treat the main crop. Only 12 to 15% production area under trap crops can be adequate for significant pest management; this characteristic makes the sorghum and sunflower mixed trap crop system very economical (Majumdar et al., 2012). In a presentation indicates that Zonosemata electa attacks many types of pepper, but has a preference for cherry pepper (Shelton & Badenes-Pérez, 2006). Shelton & Badenes-Pérez (2006) adds that Boucher et al. (2003) has reported the use of hot cherry peppers as a perimeter trap crop in bell peppers for control of pepper maggot. Rea et al. (2002) report that

in New Zealand the density of green stink bug (*Nezara viridula*) is reduced, the timing of their colonization is delayed, and cob damage to sweet corn is reduced after growing black mustard (*Brassica nigra*) around the perimeter of fields as a perimeter trap crop. Hormchan et al. (2009) report two interesting experiments conducted in Thailand, using perimeter trap cropping and row intercropping with okra and castor bean, as well as sunflower and castor bean, to track the populations of leafhopper.

Perimeter trap cropping and row intercropping of okra, and castor bean and sunflower respectively in the cotton plots have given lower numbers of cotton leafhopper and higher yields than those in the sole cotton. Shelton & Badenes-Pérez (2006) cites Pair (1997) giving an example of conventional trap cropping by using highly attractive varieties of squash to manage squash bugs and cucumber beetles in several cucurbitaceous crops. This example as perimeter trap cropping is also cited by Majumdar (2010) who points out that the squash is a suitable perimeter trap crop for the main crop-watermelon, cantaloupe and cucumber, and it is important to plant the squash earlier and apply insecticide on borders, as squash lure 66% cucumber beetles and 90% squash bugs. In a presentation Majumdar (2011), reports an example by Cook et al. (2006) for turnip rape (*Brassica rapa*) as perimeter trap crop in the main crop-oilseed rape (*B. napus*) against pollen beetles. Majumdar (2011) reports that Cook et al. (2007) call this the 'push-pull' strategy. Shelton & Badenes-Pérez (2006) explains that push-pull trap cropping is based on a combination of a trap crop (pull component), which attracts insects, with a repellent intercrop (push component) that diverts the pest away from the main crop. The so-called 'push-pull' strategy has shown very good results in Kenya. Sharma (2013) cites Linker et al. (2009), for example, Row trap crops are another option for successful control of *Heliotis* sp., as they are placed in a number of rows in the cotton, for example, every 5 rows of cotton, 1 row of sunflower or cowpea (CIKS, 2000); for every 20 rows of cotton 1 row of tobacco or corn (Hasse, 1987).

Row intercropping is the planting of a trap crop in alternating rows within the main crop

with a push-pull approach, where a trap crop is used to pull the pest species away while the protected cash crop is intercropped with a plant that repels pests. This approach has been used successfully to protect maize in Kenya. Sharma et al. (2019) cites Farstad & Jacobson (1945) with another strategy to grow wheat plants around the perimeter of a fallow field adjacent to a wheat field, with an empty space in between the fallow and wheat field, which attracts *C. cinctus* adults emerging from the previous year crop.

A strip trap crop is a specific way of arranging on one side of the crop (Parker & Snyder, 2014). Tillman (2006) points out that strip planting of trap crops is the formation along one common border between two or more crops. A well-known example of a strip trap crop is alfalfa grown to a main cotton crop to limit the development of *Lygus bug* (Majumdar, 2010; Majumdar, 2011; Meyer, 2003; PAN Germany, 2005; Rana, 2018). Shelton & Badenes-Pérez (2006) cites the authors Stern (1969) and Godfrey & Leigh (1994) who report the most widely cited example of alfalfa as a successful crop trap in conventional cotton cultivation in the central valley of California in the 1960s to control the lygus bugs. Majumdar (2011) points out that cotton, sorghum and peanuts can be effectively grown by strip trap cropping, relying on pheromone traps with high levels of parasitism reported from the tachinid fly in sorghum. Saeed et al. (2013) uses alfalfa as a strip crop, in which predators multiply, as this strip is parallel to wheat. They have found that the role of fodder crops as a trap crop in wheat is significant, as they decrease the insecticide application on the main crop. It is reported that the maximum population of aphids has been observed on plots not trap cropped with lucern, as the highest population of lice has been observed in the area without alfalfa. (Hormchan et al., 2009). In sunflower, marigold is grown to successfully control *Helicoverpa armigera*; against *Spodoptera litura* castor is grown (Sharma, 2013). Majumdar (2010) points out that alfalfa is a very suitable trap crop, which is planted in rows, and the main crop is strawberry, as it regularly vacuums an alfalfa trap crop to reduce the damage to organic strawberries by *Lygus*

*hesperus* (Swezey et al., 2007; Hagler et al., 2018). An example of the successful infestations of diamondback moth in cabbage in Florida is the use of collard greens (*Brassica oleracea* var. *acephala* L.) as a trap crop (Mitchell et al., 2000). Sarkar et al., (2018) cites Bender et al. (1999), according to who sorghum (*Sorghum bicolor* L.) has been successfully used as a trap crop in cotton fields, and according to Bukovinsky et al., (2005) black mustard reduces the kernel injury in sweet corn caused by *Nezara viridula* L.

Another effective method of planting a trap can be applied to a main cabbage crop, every 15 rows with Chinese cabbage to effectively control the cabbage webworm; mustard to effectively control the *Flea hopper*, and radishes to effectively control mustard aphid (Majumdar, 2011). Studies by Vernon et al., (2000) report that wheat planted 1 week in advance of planting strawberries is a successful trap crop because it effectively reduces wireworms (*Agriotes obscurus* L.), plant mortality and can be used as an inexpensive pest management method. Shelton & Badenes-Pérez (2006) mentions sequential trap cropping and cites the authors Pawar & Lawande (1995), who suggest that Indian mustard is suitable as a trap crop for diamondback moth, but requires planting mustard two or three times through the cabbage season because it has a shorter crop cycle than the cabbage and other cruciferous crops. According to Klopatek & Gardner (1999) a trap crop should be viewed in the larger context of landscape ecology (Shelton & Badenes- Pérez, 2006; Reddy, 2018). A trap crop is alternative IPM tactics being under investigation in New England (Boucher & Durgy, 2004; Bucher & Cheng, 2012), Tennessee (Wszelaki & Broughton, 2010), and Alabama (Majumdar et al., 2012). Tillman & Benefits of trap crops include the reduced cash crop damage, the reduction in pesticide dependence, and enhanced biodiversity (Majumdar, 2011; Majumdar et al., 2012). The advantages of trap crops in reducing the use of insecticides are highlighted: reduction of pest incidence to manageable levels (Balusu et al., 2012); conservation of indigenous natural enemies by providing shelter to be useful in the agroecosystem; growing harvested crops trap (alfalfa, squash); can be integrated with

Cottrell (2012) report scientific papers focusing on trap crops that effectively control stink bugs (Todd & Schumann, 1988) in soybean and sweet corn (Rea et al., 2002) in conventional and organic production systems. Tillman & Cottrell (2012) report that according to Wiseman & McMillian (1971) and Tillman, (2006) sorghum (*Sorghum bicolor* (L.) Moench spp. *bicolor*) is an important host plant feeding on its panicle to the stink bugs, thus suppressing *N. viridula* populations in the agricultural land in Georgia. In his publication Mertz (2018) presents the idea of the researcher Anne Nielsen's group from the Rutgers University, who has tested a combination sunflower - sorghum as a trap crop located around the perimeter of a vegetable plot to learn whether the trap crop would reduce the brown marmorated stink bug (BMSB) pressure in the vegetables. Mertz (2018) reports that according to Nielsen the Brown marmorated stink bugs are a particular problem for organic growers who cannot apply the insecticides used by traditional growers, so the use of trap crops have proven to be a solution to entice the invasive pests away from fruits. For three years Nielsen has conducted field tests on 11 organic farms in the United States to study the effectiveness of the sunflower/ sorghum trap crop, as he has indicated that BMSB could be successfully controlled. The author reports that insecticides are used once or twice per season in the trap crop to knock down BMSB; the use of pheromones increases the time the stink bugs spend on the trap crop; and the use of trap crop boost populations of samurai wasps, which would in turn reduce BMSB numbers. Another researcher, Szűcs (2019), reports that BMSB are successfully controlled in Asia by samurai wasps, and already in Michigan, samurai wasps can help control BMSB. existing farming practices (Majumdar, 2010). The shortcomings mentioned by the farmers are reported in order to clarify their use. The disadvantages of trap cropping are the need for additional planting and resources; growers need knowledge of insect behavior and migration; timely management of insects in a trap crop: otherwise you have a 'pest nursery'; and the proportion of cash crop to trap crop (Rao, 2001).

## CONCLUSIONS

Trap cropping a useful approach in farming systems. The advantages of trap crops definitely can be used in both commercial (cash crop) and non-commercial crop production. The ability to limit a pest on the farm is essential. The application of the insect trap approach has proven its efficiency in farming systems characterized with the application of pesticide treatment, but it may be also used in organic farming by using those insecticides permitted according to Regulation (EC) No 889/2008 in combination with sweep net sampling.

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## CONTROL OF MIXED WEED INFESTATION IN WINTER WHEAT

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### Abstract

During 2016-2017 and 2017-2018 a field trial with winter wheat variety "Enola" was performed. The experiment was situated on the experimental field of the Agricultural University of Plovdiv, Bulgaria. Herbicide products with mixed mode of action were evaluated - Osprey Extra, Atlantis WG, Abak and Atlantis Flex. The application of Atlantis WG – 0.50 kg ha<sup>-1</sup> + 1.00 l ha<sup>-1</sup> showed the highest control against *Anthemis arvensis*, *Papaver rhoeas* and *Sinapis arvensis*. The highest efficacy against the weeds *Lamium purpureum* and *Avena fatua* after the application of Abak + Mero – 0.25 kg ha<sup>-1</sup> + 1.00 l ha<sup>-1</sup> was reported. The treatment with Osprey Extra + Biopower – 0.33 kg ha<sup>-1</sup> + 1.00 l ha<sup>-1</sup> showed highest efficacy against *Lolium rigidum* and *Galium aparine*. The most difficult-to-control weeds were the volunteer of Clearfield® oilseed rape and *Veronica hederifolia*. The height of the plants, length of the central ear, average number of grains per ear, average grain weight per ear, 1000 grain weight and yields were the highest after the treatment with Atlantis WG – 0.50 kg ha<sup>-1</sup> + Biopower – 1.00 l ha<sup>-1</sup> and Abak – 0.25 kg ha<sup>-1</sup> + Mero – 1.00 l ha<sup>-1</sup>.

**Key words:** winter wheat, biometry, herbicides, weeds, efficacy introduction.

### INTRODUCTION

The winter wheat (*Triticum aestivum* L.) is main grain crop in Bulgaria. The total harvested area for 2019 are 1 198 682 ha with average yields of 5.141 t ha<sup>-1</sup> (MZH, 2020). The weeds are main competitors of winter wheat for water, nutrients and light. The weeds also cause indirect damage, as many of the species are hosts of diseases and pests (Kalinova et al., 2012). The high weeds infestation can decrease the yields up to 70% (Atanasova and Zarkov, 2005; Bekelle, 2004). In the modern agriculture the weed control is mainly performed by the chemical method. A number of authors study the selectivity and efficacy of different herbicides in crops (Marinov-Serafimov and Golubinova, 2016; Marinov-Serafimov and Golubinova, 2015; Rankova and Tityanov, 2015; Rankova et al., 2014; Goranovska and Kalinova, 2014; Hristova, 2007; Atanasova, 2002). The choice of a proper herbicide, optimal time and application rate are one of the most important and responsible moments in the wheat management (Petrova, 2017; Penchev and Petrova, 2015; Petrova and Sabev, 2014; Abbas et al., 2009a; Khalil et al., 2008; Sherawat et al., 2005). For graminaceous weeds

control in wheat Raffel et al. (2010) recommend application of Tarox (pinoxaden + clodinafop-propargyl). Berca and Horoias (2013) conduct a trial for *Avena fatua* control. The researchers reported that at very high density of the weed - from 600 to 900 specimens per m<sup>2</sup>, very good results after the application of Palas 75 WG in rate of 150 g ha<sup>-1</sup>. For *Alopecurus myosuroides* control in wheat Kierzek et al. (2006) recommend the application of pinoxaden in tank mix with adjuvant in 2<sup>nd</sup> - 4<sup>th</sup> leaf stage of the crop and in 1<sup>st</sup> - 4<sup>th</sup> leaf stage of the weed. It was found that in the crop rotation of winter wheat with winter oilseed rape the application of propyzamide and pyroxulam the density of *Alopecurus myosuroides* was decreased (Roberts and Jackson, 2012).

For successful control of the resistant to herbicides *Alopecurus myosuroides* in wheat the application soil herbicides in the autumn followed by spring application of mesosulfuron + iodosulfuron is applied (Atlantis) (Gehring and Thyssen, 2014).

It is economically unreasonable to use herbicides for control of graminaceous weeds without performing successful control of broadleaf weeds. Mitkov et al. (2017) study Sekator OD and Biathlon 4 D for control of

dicotyledonous weeds. The herbicide products were applied in two terms – 1<sup>st</sup> - 2<sup>nd</sup> stem node (BBCH 30-32) and flag leaf (BBCH 37-39) of the wheat. The highest efficacy was recorded after the application of Biathlon 4 D + Dash in rate of 0.14 kg ha<sup>-1</sup> + 1.0 l ha<sup>-1</sup> applied 1<sup>st</sup> – 2<sup>nd</sup> stem node of the crop. Chopra et al. (2008) found that carfentrazone in rate of 20 g ha<sup>-1</sup> and metsulfuron in rate of 4 g ha<sup>-1</sup> control the broadleaf weeds 83.7 and 84.1% respectively. For control of the broadleaf weeds Abbas et al. (2009b) recommend the usage of Buktril Super EK - 835 ml ha<sup>-1</sup> and Starane-M - 875 ml ha<sup>-1</sup>. WangCang et al. (2016), reported that the combinations of 29% fluroxypyr - 111.31 g ha<sup>-1</sup> + 5% carfentrazone-ethyl - 3.31 g ha<sup>-1</sup>, florasulam - 7.50 g ha<sup>-1</sup> + carfentrazone-ethyl - 15.00 g ha<sup>-1</sup> had excellent efficacy against *Descurainia sophia*, *Capsella bursa-pastoris* and *Galium aparine*. For the most efficient control of *Galium* sp. fluroxypyr should be applied. High efficacy against *Galium aparine* after the combine treatment of carfentrazone + MCPP, triosulfuron + dicamba, and amidosulfuron + iodosulfuron was recorded (Cirujeda et al., 2008).

Besides differentiated, the control of the mono- and dicotyledonous weeds in wheat could be performed simultaneously. For *Apera spica-venti*, *Echinochloa crus-galli* and some dicotyledonous weeds, Szemendera et al. (2008) found that Pledge 50 WP (flumioxazine) can be applied. If there is mixed weed infestation with *Apera spica-venti*, *Lolium* sp., *Avena fatua*, *Myosotis arvensis*, *Capsella bursa-pastoris*, *Thlaspi arvense*, etc. Krato and Raffel (2018) observed that treatment with Avoxa (pinoxadene+pyroxulam + cloquintocet-mexyl) in rate of 1.8 l ha<sup>-1</sup> may be performed. The aim of the study is to establish the possibilities for chemical control of mixed weed infestation in winter wheat.

## MATERIALS AND METHODS

During the experimental seasons of 2016-2017 a field trial with winter wheat variety “Enola” was performed. The experiment was situated on the experimental field of the department of “Agriculture and herbology” at the Agricultural University of Plovdiv, Bulgaria. The trial was

conducted by the randomized block design in 3 replications. The size of the harvesting plot was 20 m<sup>2</sup>. Variants of the trial were: 1. Untreated control; 2. Osprey Extra (mesosulfuron-methyl + thien carbazon-methyl + mefenpyr-diethyl) - 166 g ha<sup>-1</sup> + Biopower (adjuvant) - 1000 ml ha<sup>-1</sup>; 3. Osprey Extra – 200 g ha<sup>-1</sup> + Biopower - 1000 ml ha<sup>-1</sup>; 4. Osprey Extra - 255 g ha<sup>-1</sup> + Biopower - 1000 ml ha<sup>-1</sup>; 5. Osprey Extra - 333 g ha<sup>-1</sup> + Biopower - 1000 ml ha<sup>-1</sup>; 6. Osprey Extra - 330 g ha<sup>-1</sup>; 7. Atlantis WG (iodosulfuron-methyl-sodium+ mesosulfuron-methyl + thien carbazon-methyl) 500 g ha<sup>-1</sup> + Biopower - 1000 ml ha<sup>-1</sup>; 8. Abak (pyroxsulame + cloquintocet-mexyl) - 250 g ha<sup>-1</sup> + Mero (adjuvant) - 1000 ml ha<sup>-1</sup>; 9. Atlantis Flex (mesosulfuron-methyl + propoxycarbazone-natrium + mefenpyr-diethyl) - 330 g ha<sup>-1</sup> + Biopower - 1000 ml ha<sup>-1</sup>. Predecessor of winter wheat during both experimental years was Clearfield® winter oilseed rape (*Brassica napus* L.).

To the whole experimental area before sowing of the winter wheat, fertilization with 300 kg ha<sup>-1</sup> with NPK (15:15:15) 300 kg ha<sup>-1</sup> NH<sub>4</sub>NO<sub>3</sub> as spring dressing.

Artificial weed infestation with previously collected weed seeds by spreading to the whole area of the trial was performed. The infestation was done with *Anthemis arvensis* L., *Papaver rhoeas* L., *Galium aparine* L., *Sinapis arvensis* L., *Lamium purpureum* L., *Veronica hederifolia* L., *Lolium rigidum* L., *Avena fatua* L., and volunteer Clearfield® *Brassica napus* L. The application of the herbicide products was done in beginning of spindling stage of the crop (BBCH 30-31) with spraying solution 250 l ha<sup>-1</sup>. The efficacy of the herbicides was evaluated on the 14<sup>th</sup>, 28<sup>th</sup>, and on the 56<sup>th</sup> day after treatments by the 10-score scale of EWRS. The selectivity of the herbicides was examined on the 7<sup>th</sup>, 14<sup>th</sup>, 28<sup>th</sup> and 56<sup>th</sup> day after application by the 9-score scale of EWRS (Zhelyazkov et al., 2017).

The following parameters of the winter wheat were determined: Height of the plants at the end of the vegetation, length of the dental ear, number of grains per ear, mass of the grain per ear, 1000 grain weight and grain yield. The obtained data were processed by the statistical software SPSS 19.

## RESULTS AND DISCUSSIONS

On the experimental field only annual weeds during both vegetation seasons of the winter wheat were observed. The weeds were presenters of three biological weed groups. The highest population was from the group of the early-spring weeds - *Galium aparine* L., *Sinapis arvensis* L., *Lamium purpureum* L. and *Avena fatua* L., followed by the group of the winter spring weeds - *Anthemis arvensis* L., *Papaver rhoeas* L. and *Lolium rigidum* L. and one presenter of the ephemerals - *Veronica hederifolia* L.

On the field the Clearfield® oilseed rape volunteer *Brassica napus* L. was also reported. The efficacy of the studied herbicide products

is presented on average for both experimental years on Tables 1, 2 and 3.

The highest efficacy 14 days after herbicide application against *A. arvensis* (38.3%), *P. rhoeas* (30.0%), *G. aparine* (35.0%), *S. arvensis* (58.3%) and *L. purpureum* (46.6%) for Atlantis WG (500 g ha<sup>-1</sup> + Biopower - 1000 ml ha<sup>-1</sup>) was reported.

Lower efficacy was reported against *G. aparine*, *S. arvensis* and *L. purpureum* after the application of Atlantis Flex - 330 g ha<sup>-1</sup> + Biopower - 1000 ml ha<sup>-1</sup> (variant 9). The efficacy obtained was 31.6%, 35.0% and 31.6% respectively. For the treatment with Abak - 250 g ha<sup>-1</sup> + Mero - 1000 ml ha<sup>-1</sup> the efficacy against *A. arvensis* was only 25% average for both years of the study.

Table 1. Efficacy of the studied herbicide products on the 14<sup>th</sup> day after application average for 2017 and 2018, %

Treatments	<i>A. arvensis</i>	<i>P. rhoeas</i>	<i>G. aparine</i>	<i>S. arvensis</i>	<i>V. hederifolia</i>	<i>L. purpureum</i>	<i>L. rigidum</i>	<i>A. fatua</i>	<i>B. napus</i> CL
1. Untreated contol	-	-	-	-	-	-	-	-	-
2. Osprey Extra - 166 g ha <sup>-1</sup> + Biopower - 1000 ml ha <sup>-1</sup>	6.6	10.0	10.0	21.6	13.3	10.0	8.3	0.0	0.0
3. Osprey Extra - 200 g ha <sup>-1</sup> + Biopower - 1000 ml ha <sup>-1</sup>	10.0	11.6	13.3	28.3	11.6	21.6	11.6	0.0	0.0
4. Osprey Extra - 255 g ha <sup>-1</sup> + Biopower - 1000 ml ha <sup>-1</sup>	10.0	11.6	13.3	25.0	11.6	20.0	16.6	5.0	0.0
5. Osprey Extra - 333 g ha <sup>-1</sup> + Biopower - 1000 ml ha <sup>-1</sup>	25.0	25.0	21.6	28.3	18.3	26.6	10.0	1.6	0.0
6. Osprey Extra - 330 g ha <sup>-1</sup>	18.3	21.6	25.0	30.0	20.0	26.6	5.0	5.0	0.0
7. Atlantis WG - 500 g ha <sup>-1</sup> + Biopower - 1000 ml ha <sup>-1</sup> ;	38.3	30.0	35.0	58.3	25.0	46.6	8.3	3.3	0.0
8. Abak - 250 g ha <sup>-1</sup> + Mero - 1000 ml ha <sup>-1</sup>	25.0	0.0	5.0	26.6	10.0	21.6	10.0	5.0	0.0
9. Atlantis Flex - 330 g ha <sup>-1</sup> + Biopower - 1000 ml ha <sup>-1</sup>	21.6	20.0	31.6	35.0	30.0	31.6	8.3	5.0	10.0

On the first reporting date the highest efficacy against *V. hederifolia* after the application of Atlantis Flex - 330 g ha<sup>-1</sup> + Biopower - 1000 ml ha<sup>-1</sup> - 30.0% average for the period was observed.

The efficacy against *L. rigidum* and *A. fatua* was very low after the treatments of all herbicide products. The Clearfield oilseed rape volunteer (*B. napus*) was the most difficult-to-

control weed in our experiment. Only 10.0% efficacy was recorded after the application of Atlantis Flex - 330 g ha<sup>-1</sup> + Biopower - 1000 ml ha<sup>-1</sup> (Table 1).

In previous our study the Clearfield® oilseed rape volunteer also appeared to be a very resistant to chemical control “weed”. The highest efficacy for the conditions of the study after the treatment with thifensulfuron-methyl

+ fluroxypyr (750 ml ha<sup>-1</sup>) was recorded - 40% (Mítkov et al., 2018).

On the second reporting date the highest efficacy against *A. arvensis*, *P. rhoeas*, *S. arvensis* and *L. purpureum* again from Atlantis WG (500 g ha<sup>-1</sup> + Biopower - 1000 ml ha<sup>-1</sup>) was found - 86.6%, 88.3%, 91.6% and 90.0% respectively (Table 2). Efficacy reaching 90.0% against of *L. purpureum* for the treatment of

Abak - 250 g ha<sup>-1</sup> + Mero - 1000 ml ha<sup>-1</sup> was observed. For *G. aparine* control, the highest results for the treatment with Osprey Extra - 333 g ha<sup>-1</sup> + Biopower - 1000 ml ha<sup>-1</sup> was reported. Against *V. hederifolia* and *B. napus* the highest efficacy on the 28<sup>th</sup> day after treatments with Atlantis Flex - 330 g ha<sup>-1</sup> + Biopower - 1000 ml ha<sup>-1</sup> - 50 and 23.3% respectively was observed (Table 2).

Table 2. Efficacy of the studied herbicide products on the 28<sup>th</sup> day after application average for 2017 and 2018, %

Treatments	<i>A. arvensis</i>	<i>P. rhoeas</i>	<i>G. aparine</i>	<i>S. arvensis</i>	<i>V. hederifolia</i>	<i>L. purpureum</i>	<i>L. rigidum</i>	<i>A. fatua</i>	<i>B. napus</i> CL
1. Untreated contol	-	-	-	-	-	-	-	-	-
2. Osprey Extra - 166 g ha <sup>-1</sup> + Biopower - 1000 ml ha <sup>-1</sup>	45.0	53.3	35.0	73.3	18.3	60.0	31.6	35.0	0.0
3. Osprey Extra - 200 g ha <sup>-1</sup> + Biopower - 1000 ml ha <sup>-1</sup>	58.3	63.3	55.0	81.6	26.6	76.6	41.6	53.3	0.0
4. Osprey Extra - 255 g ha <sup>-1</sup> + Biopower - 1000 ml ha <sup>-1</sup>	56.6	70.0	65.0	81.6	28.3	85.0	48.3	56.6	0.0
5. Osprey Extra - 333 g ha <sup>-1</sup> + Biopower - 1000 ml ha <sup>-1</sup>	60.0	83.3	78.3	85.0	38.3	85.0	61.6	68.3	0.0
6. Osprey Extra - 330 g ha <sup>-1</sup>	70.0	80.0	66.6	86.6	26.6	85.0	50.0	65.0	0.0
7. Atlantis WG - 500 g ha <sup>-1</sup> + Biopower - 1000 ml ha <sup>-1</sup> ;	86.6	88.3	71.6	91.6	38.3	90.0	63.3	70.0	0.0
8. Abak - 250 g ha <sup>-1</sup> + Mero - 1000 ml ha <sup>-1</sup>	78.3	0.0	45.0	90.0	20.0	90.0	71.6	83.3	0.0
9. Atlantis Flex - 330 g ha <sup>-1</sup> + Biopower - 1000 ml ha <sup>-1</sup>	63.3	75.0	41.6	81.6	50.0	78.3	58.3	70.0	23.3

The efficacy on the 56<sup>th</sup> day after treatments kept the tendency observed on the first two reporting dates.

It is worth noting that the herbicide efficacy was the highest on the third reporting date.

Atlantis WG - 500 g ha<sup>-1</sup> + Biopower - 1000 ml ha<sup>-1</sup> showed the highest results against *A. arvensis*, *P. rhoeas* and *S. arvensis* - 96.6, 91.6 and 100%, respectively.

Against *S. arvensis* 100% efficacy after the spraying with Abak - 250 g ha<sup>-1</sup> + Mero - 1000 ml ha<sup>-1</sup> followed by treatments 5, 6 and 9 where the efficacy reached 98.3% (Table 3). Excellent efficacy against *L. purpureum* and *A. fatua* of

Abak - 250 g ha<sup>-1</sup> + Mero - 1000 ml ha<sup>-1</sup> was observed - 100% and 98.3%, respectively.

The highest efficacy against *L. rigidum* and *G. aparine* on the 56<sup>th</sup> day after application for the treatment with Osprey Extra - 333 g ha<sup>-1</sup> + Biopower - 1000 ml ha<sup>-1</sup>, 93.3% and 86.6% respectively was recorded.

During both experimental years the lowest and unsatisfactory efficacy was observed for the volunteer *B. napus*. Among all treatments with the exception of Atlantis Flex - 330 g ha<sup>-1</sup> + Biopower - 1000 ml ha<sup>-1</sup> the control was 0% (Table 3).

Table 3. Efficacy of the studied herbicide products on the 56<sup>th</sup> day after application average for 2017 and 2018, %

Treatments	<i>A. arvensis</i>	<i>P. rhoeas</i>	<i>G. aparine</i>	<i>S. arvensis</i>	<i>V. hederifolia</i>	<i>L. purpureum</i>	<i>L. rigidum</i>	<i>A. fatua</i>	<i>B. napus CL</i>
1. Untreated contol	-	-	-	-	-	-	-	-	-
2. Osprey Extra - 166 g ha <sup>-1</sup> + Biopower - 1000 ml ha <sup>-1</sup>	50.0	60.0	45.0	85.0	24.0	80.0	78.3	90.0	0.0
3. Osprey Extra - 200 g ha <sup>-1</sup> + Biopower - 1000 ml ha <sup>-1</sup>	63.3	70.0	61.6	95.0	38.3	95.0	90.0	95.0	0.0
4. Osprey Extra - 255 g ha <sup>-1</sup> + Biopower - 1000 ml ha <sup>-1</sup>	68.3	76.6	68.3	96.6	45.0	96.6	88.3	95.0	0.0
5. Osprey Extra - 333 g ha <sup>-1</sup> + Biopower - 1000 ml ha <sup>-1</sup>	83.3	91.6	86.6	98.3	55.0	98.3	93.3	91.6	0.0
6. Osprey Extra - 330 g ha <sup>-1</sup>	83.3	81.6	73.3	98.3	55.0	98.3	91.6	93.3	0.0
7. Atlantis WG - 500 g ha <sup>-1</sup> + Biopower - 1000 ml ha <sup>-1</sup> ;	96.6	91.6	83.3	100	46.6	98.3	90.0	96.6	0.0
8. Abak - 250 g ha <sup>-1</sup> + Mero - 1000 ml ha <sup>-1</sup>	95.0	0.0	78.3	100	45.0	100	90.0	98.3	0.0
9. Atlantis Flex - 330 g ha <sup>-1</sup> + Biopower - 1000 ml ha <sup>-1</sup>	85.0	75.0	70.0	98.3	63.3	96.6	81.6	96.6	50.0

The boxplot of the biometrical data is presented on Figure 1. Regarding the indicator central stem height we found that the plants from treatment 7 (Atlantis Flex + Biopower - 500 g + 1000 ml ha<sup>-1</sup>) were the highest. From the figure is seen that the stem height vary in the

narrow extend, so we concluded that the plans of the concrete treatment were the most uniformed. According to the next parameter - the central ear length, with the highest and uniformed values were the plants from treatment 7 also.

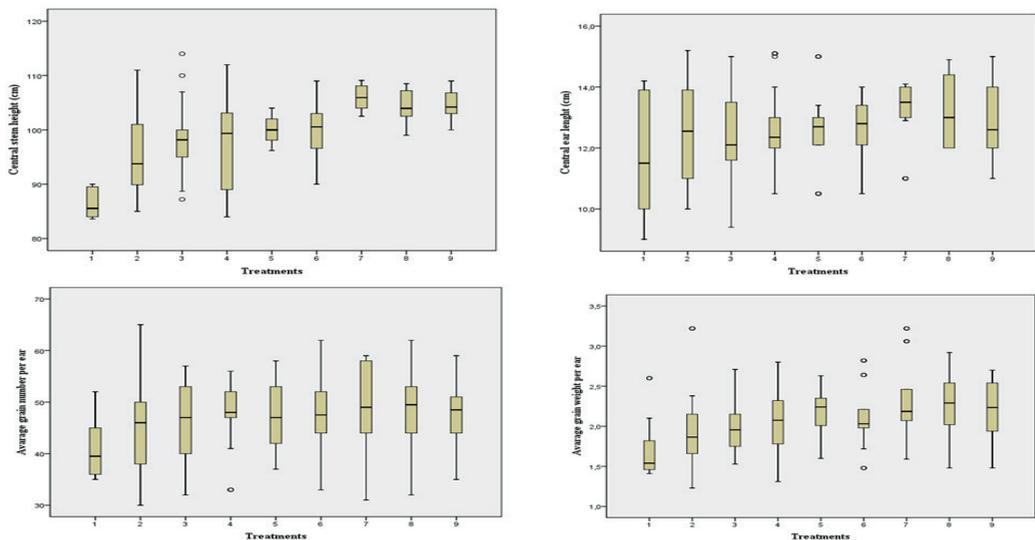


Figure 1. Boxplot of the biometrical data

The highest grain number per ear was found for treatment 7, and the lowest for the untreated control was recorded. The average grain weight per ear was for the treatments 5, 8, 9 and 7.

The results regarding the biometrical parameters and yields are presented on Table 4. For the indicator central ear length, statistical difference in the results between the untreated control and the treated variants were found. The lowest values for the untreated control - 11.86 cm were found, and the highest - 13.28 cm for the treatment Atlantis WG + Biopower. For the parameter average grain number per ear, statistically proved differences for the treatments with herbicide application were found. The average grain number per ear varied between 46.43 to 48.90. Lower average grain number per ear was recorded for treatment 2 (Osprey Extra + Biopower)– 45.00.

The untreated control had the lowest average grain weight per ear - 1.70 g. This result is statistically proven at a level of significance of 5%. The highest average grain weight per ear for treatment 7 was found.

The results for the 1000 grain weight showed that there is statistically proved difference between the treated variants and the untreated control. The untreated control had the lowest results - 38.23 g, and the highest for treatment 7 was recorded - 46.20 g.

Data on wheat yield confirms that there is a positive correlation between the effect of herbicides on weeds and the yield obtained from the crop. As a result from the high weed infestation very low average yield for the untreated control was achieved - 2.54 t ha<sup>-1</sup>. At treatments 7 and 8 the highest yield was obtained - 5.15 t ha<sup>-1</sup> and 5.05 t ha<sup>-1</sup> respectively. The variants 2, 3 and 4 treated with Osprey Extra in rates of 166, 200 250 g ha<sup>-1</sup> Biopower - 1000 ml ha<sup>-1</sup> also deserve attention. In these treatments, the increase in yield compared to the untreated control is also statistically proved. The lower results regarding the yields of these treatments could be explained with the lowest efficacy of the herbicide product applied in the concrete rates.

Table 4. Parameters and yields of the winter wheat, average for 2017 and 2018

Treatments	Central stem height, cm	Central ear length, cm	Average grain number per ear	Average grain weight per ear, g	1000 grain weight, g	Yield, t ha <sup>-1</sup>
1.	86.43 a	11.86 a	41.23 a	1.70 a	38.23 a	2.54 a
2.	96.10 b	12.53 b	45.00 b	1.94 b	40.12 b	3.31 b
3.	97.89 bcd	12.54 b	46.43 bc	2.00 bc	41.72 c	3.54 bc
4.	97.43 bc	12.57 b	47.50 bc	2.08 bcd	42.18 cd	3.80 c
5.	100.01 d	12.67 bc	47.10 bc	2.17 cde	42.96 ef	4.57 d
6.	99.72 cd	12.65 bc	47.10 bc	2.10 bcd	42.62 de	4.48 d
7.	105.91 e	13.28 c	48.70 bc	2.32 e	46.20 h	5.15 e
8.	104.48 e	13.18 bc	48.90 c	2.27 de	44.14 g	5.05 e
9.	104.36 e	12.97 bc	47.50 bc	2.19 cde	43.66 fg	4.60 d
	$gD_{5\%} = 2.5512$	$gD_{5\%} = 0.6675$	$gD_{5\%} = 3.7087$	$gD_{5\%} = 0.2010$	$gD_{5\%} = 0.7436$	$gD_{5\%} = 44.6512$

## CONCLUSIONS

Atlantis WG + Biopower in rates of 500 g ha<sup>-1</sup> + 1000 ml ha<sup>-1</sup> showed the highest efficacy against the weeds *Anthemis arvensis*, *Papaver rhoeas* and *Sinapis arvensis*.

The highest herbicide control of *Lamium purpureum* and *Avena fatua* was achieved after the application of Abak + Mero in rates of 250 g ha<sup>-1</sup> + 1000 ml ha<sup>-1</sup>.

Osprey Extra + Biopower applied in rates of 333 g ha<sup>-1</sup> + 1000 ml ha<sup>-1</sup> showed good efficacy against *Lolium rigidum* and *Galium aparine*.

The most difficult-to-control-weeds were the volunteer Clearfield *Brassica napus* and *Veronica hederifolia*. The highest efficacy against these weeds on the 56<sup>th</sup> day after application of Atlantis Flex + Biopower in rates of 330 g ha<sup>-1</sup> + 1000 ml ha<sup>-1</sup> - 50 and 63.3% respectively was reported.

There were no visible signs of phytotoxicity to the crop caused by any of the examined herbicide products.

The lowest results regarding the central stem height, central ear length, Average grain number per ear, average grain weight per ear,

1000 grain weight as well as the yield for the untreated control was found. Statistical differences have been identified in favour of herbicide-treated variants.

The highest results for all evaluated biometrical parameters as well as yield after the application of Atlantis WG + Biopower in rates of 500 g ha<sup>-1</sup> + 1000 ml ha<sup>-1</sup> and Abak + Mero in rates of 250 g ha<sup>-1</sup> + 1000 ml ha<sup>-1</sup> were obtained

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## IMPACT OF BIOFERTILIZERS ON VEGETATIVE GROWTH AND SOIL RESPIRATION IN *Triticum spelta* L. GROWN IN ORGANIC FARMING

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### Abstract

*Intensive agricultural production alters the natural cycle of nutrients in the soil, very often leading to a reduction in organic matter and soil degradation. The use of biofertilizers would solve these problems and make the ecosystem healthier. The biofertilizers used in the study have a positive effect on *Triticum spelta* L. growth. The results of the treated variants with Litovit, Tryven, Baikal EM and Amalgerol, show a higher height of the plants in the stem elongation phase compared to the control, by 20%, 15%, 12% and 10.8%, respectively. At full ripeness, the general stimulating effect of the applied foliar biofertilizers on the height, better nutrition and growth of the plants is taken into account. Greater soil microbial activity in the studied biofertilizers was observed on the 14<sup>th</sup> day after treatment. Higher values are reported for all variants of combined fertilization (foliar fertilization on basic fertilization with Agriorgan pellet) compared to unfertilized control and self-fertilization with Agriorgan pellet, which is statistically proven.*

**Key words:** biofertilizers, organic agriculture, *Triticum spelta* L., soil respiration, vegetative growth.

### INTRODUCTION

Agriculture alters the natural cycling of nutrients in soil (Mishra & Dash, 2014) especially when there is an intensification in agriculture which has a negative impact to agriculture ecosystem that is including decrease of organic matter (Altuhaish & Tjahjoleksono, 2014), export of nutrients (Minev et al., 2019) and soil degradation. Organic farming methods (such as the use of biofertilizers) would solve these issues and make the ecosystem healthier (Mishra & Dash, 2014). Biofertilizers has been identified as an alternative to chemical fertilizers to increase soil fertility and crop production in sustainable farming (Mazid et al., 2011b; Mishra et al., 2015; Javoreková et al., 2015). The biofertilization was beneficial in stimulating the growth for plants (Rivera-Cruz et al., 2008; Abd El- Gleel Mosa et al., 2018). Many authors point out that energy renewables in the agro-ecosystem, food security and the sustainability of agriculture depends on healthy and fertile soil (Singh et al., 2017; Sabbagh et al., 2017). This is achieved by applying environmental friendly source of plant nutrient, such as biofertilizers (Bhattacharjee & Dey, 2014). Javoreková et al. (2015) and Mazid et al. (2011a) points out that according to Vessey,

(2003) biofertilizer as a substance which contains living microorganisms which, when applied to seed, plant surfaces or soil, colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant. Biofertilizers play an important role in improving nutrient supplies for crops (Pal et al., 2015) and hold a great promise to improve crop yields through environmentally better nutrient supplies (Wu et al., 2005). Mazid & Khan (2014) points out that according to Ghosh (2004) liquid biofertilizers are special liquid formulations containing not only the desired microorganisms and their nutrients but also special cell protectants or chemicals that promote formation of resting spores or cysts for longer shelf life and tolerance to adverse conditions. Agricultural practices that improve soil quality and agricultural sustainability have received much attention by researchers and farmers. The role of organic manures in plant nutrition is now attracting the attention of agriculturists and soil scientists throughout the world (Jat et al., 2015). Organic manures releasing nutrients rather slowly and steadily over a longer period (Makinde et al., 2007) and can be used to promote the healthy population of beneficial organisms in the soil (Rather et

al., 2018). Soil organisms are very important for the management of soil fertility in agriculture (Bing-Ru et al., 2006) and microorganisms that perform important functions in the soil (Rizvanov et al., 1988; Araújo & Monteiro, 2006). Soil microbial respiration is the most characteristic indicator for determining soil microbial activity (Tu et al., 2006). Soils that maintain a high content of microbial biomass have an accumulation of nutrients that are involved in the cycle of the soil system (Gregorich, 1994).

Spelled wheat (*Triticum spelta* L.) belongs to the group of hexaploid wheat, and until the beginning of the XX<sup>th</sup> century it was the predominant cereal and fodder crop in many regions of Southwest Germany (Blatter et al., 2004), as well as in parts of Switzerland and Austria (Poltoretskyi et al., 2018). Today it is a relic culture in Central Europe and northern Spain. As it has a number of valuable properties - it improves blood circulation and digestion, facilitates kidney function, finds its prestigious place as a healthy food (Kohajdová & Karovičová, 2008; Arzani & Ashraf, 2017) and although low yield is very suitable for preparation of pasta because it contains a balanced amount of gluten, which further enhances the interest in this crop grown especially in the system of organic production (Ugrenović et al., 2018). The aim is to study the influence of the applied biofertilizers on the vegetative growth and soil respiration of *Triticum spelta* L., in the conditions of a plant-growing organic farm.

## MATERIALS AND METHODS

The study was conducted during 2014-2017 at the Agroecological Center at the Agricultural University, Bulgaria. The following factors were studied: Vegetation year (2014/2015; 2015/2016; 2016/2017); Wheat species - *Triticum spelta* L.; Fertilizers - Agriorgan pellet biofertilizer in dose 100 kg/da is used as a basic fertilization of the whole experimental area and the Amalgerol, Lithovit, Baikal EM and Tryven as foliar application in the different variants. All foliar fertilizers are applied twice in the tillering and the stem elongation phases in the following concentrations, respectively: Amalgerol- 200 ml/da in tillering phase and

500 ml/da in the stem elongation phase; Lithovit- in a dose of 150 g/da; Baikal EM - with 0.1% solution; Tryven- 400 ml/da. The soil of the Agroecological Center is alluvial-meadow (Mollic Fluvisols in the FAO) classification, with a slightly alkaline reaction, and the profile is represented by a humus horizon with a thickness of 10 to 40 cm, below which follow alluvial deposits unaffected by soil formation. Alluvial-meadow soils are loose, ventilated and warm. Most of these soils are characterized by high fertility on the soil type (Popova & Sevov, 2010).

### Characteristics of used biofertilizers:

**Amalgerol**- is a liquid emulsion concentrate rich in hydrocarbons and natural plant growth hormones. Contains seaweed extracts, distilled paraffin oil, vegetable oils, distilled herbal extracts. **Lithovit**- is a high quality nanotechnology product created by tribodynamic activation and micronization. Contains (CaCO<sub>3</sub>) - 79.19%; (MgCO<sub>3</sub>) - 4.62%; (Fe) - 1.31% and others. **Baikal EM** - is a probiotic product containing beneficial microorganisms (lactic acid bacteria, yeast, bifidobacteria, enzymes and spore bacteria) which are antagonists of pathogenic and opportunistic microflora. **Tryven** - is a complex mixture of NPK, contains (N)- 24.4%; Organic nitrogen- 17.3%; (P<sub>2</sub>O<sub>5</sub>)- 17.2%; (K<sub>2</sub>O)- 7.42%. It has a good systemic effect, especially nitrogen. **Agriorgan pellet** - is an organic fertilizer from sheep manure, enriched with microorganisms and a supplement of trace elements. Contains: Organic nitrogen (N) - 2.5%; (P<sub>2</sub>O<sub>5</sub>) - 3.0%; (K<sub>2</sub>O)- 1.0%; Organic carbon (C) - 28.5%; Humic acids - 6.0%; pH - 6, fulvic acids.

**Agrotechnics of experience.** Agriorgan pellet fertilizer in a dose of 100 kg/da is imported in the soil at depth of 10-15 cm together with crushing of the pepper predecessor plant biomass and mixing with the soil. Plant residues enrich the soil with organic matter necessary for soil organisms and maintaining soil fertility. Against late autumn and early spring weeds all the experimental area was harrowed with harrow, in the early spring in March when the crop is in tillering phenophase and the weeds are in their early development. This method is extremely successful for

controlling weeds in cereals for organic production.

**Study Parameters: Plant height** - measure the height of 10 plants of each variant and each repetition in the phases tillering, stem elongation, full ripenes.

**'Soil respiration'** - Total microbiological activity is determined by the amount of CO<sub>2</sub> released (determination of the intensity of CO<sub>2</sub> release). The method used is a modification of the method of Stotzky (1965) (Sapundzhieva et al., 2010).

The statistical processing of the experimental data was performed using SPSS V. 9.4 for Microsoft Windows (SAS Institute Inc., 1999), by Duncan, Anova. A Duncan multiple-range test was also performed to identify the homogeneous type of the data sets among the different treatments at P<0.05 level (Duncan, 1955).

## RESULTS AND DISCUSSIONS

**1. Agroclimatic characteristics.** The vegetation year 2014-2015 is characterized as relatively warm, and precipitation is above normal, except January and April, which defines the year as relatively humid. After sowing in abundant rainfall conditions in the third ten days (116 mm/m<sup>2</sup>), combined with temperatures above the norm (12.8°C) allowed timely emergence of plants. The winter period is characterized with high rainfall values and temperatures, which is good for all the processes, related to the plant cold resistance good enter the tillering phenophase, and successfully overwinter. The spring vegetation is in heavy rainfall conditions (138 mm/m<sup>2</sup>), 94 mm/m<sup>2</sup> above the norm, which is with positive effect on crop development in the tillering and stem elongation phenophases. During May and June, when wheat is heading, temperatures and precipitations are found in values above the norm (Table 1).

The vegetation year 2015-2016 is characterized as relatively warm, with temperatures above the norm and relatively humid. The heavy rainfall in October (70.3 mm/m<sup>2</sup>) contributes to a good supply of soil with moisture for normal crop

germination. Temperatures in the period December-February are above the norm, which allows successful overwintering of the plants. From March, the temperature values are slightly above the norm, precipitation in May (64.7 mm/m<sup>2</sup>) twice over the long-term period, which gives its positive attitude to the plants development (Table 2). 2016-2017 is characterized as relatively warm with uneven precipitation. The beginning of crop vegetation in the autumn takes place under favorable conditions, characterized by temperature values close to the long-term period. The renewed vegetation in March is characterized by temperatures above the norm (by 3.7°C) and precipitation above the norm of 47.9 mm/m<sup>2</sup>, favorable for the crop development.

**2. Plant height, cm.** The influence of the foliar biofertilizers on *Triticum spelta* plants height is presented on Table 3. In 2015, during tillering phase, higher values were reported in the variants treated with Lithovit and Amalgerol, respectively 12.87 cm and 12.80 cm. The tendency for higher plant is maintained in the stem elongation phase with the same treatment options, with values of 60.67 cm and 58.33 cm, and in full ripenes when treated with biofertilizer Baikal EM- 84.35 cm, followed by the variant treated with Lithovit and Agriorgan pellets.

In 2016, during the tillering phase, the values differ, but close to the control. In the stem elongation phase, is observed positive effect on the plant height at combined foliar and soil fertilization in all variants, compared to the control plants. It is announced with unfavorable conditions for nutrients absorption in February and March - high air temperatures and low precipitation values. The highest plants were reported at Lithovit treated variants (21%), Tryven and Baikal EM (11% above the control). This confirms the effectiveness of foliar fertilization as a complementary element in plant nutrition. At full ripenes, higher values of the indicator were reported in the variants, treated with Amalgerol - 87.52 cm (5% above control) and Tryven - 87.15 cm (4% above control).

Table 1. Average daily air temperatures by ten days (°C), 2014-2017

Months	IX			X			XI			XII			I			II			III			IV			V			VI		
	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
Ten days	20.7	18.6	14.8	14.1	15.2	9.2	8.8	10.2	4.8	6.7	4.3	4.4	0.5	2.4	6.5	3.3	0.8	6.9	4.7	5.5	9.9	7.9	14.8	14.6	19.3	19.3	19.4	20.9	22.7	19.6
Av. monthly t°C	18.1			12.8			7.9			5.1			3.1			3.7			6.7			12.4			19.3			21.1		
2015/16	22.9	20.3	18.4	14.9	14.5	9.2	9.6	13.2	11.2	7.1	3.4	4.8	0.1	1.5	-2.0	5.1	9.3	9.7	10.0	7.2	10.6	15.4	17.4	13.7	14.1	16.8	20.1	19.8	24.3	25.6
Av. monthly t°C	21			12.8			11.3			5.1			-0.1			8.0			9.3			15.5			17.0			23.3		
2016/17	21.8	21.7	15.2	14.9	11.2	12.1	10.3	5.6	3.9	4.1	0.4	2.0	-4.4	-2.0	-5.4	-0.8	1.4	9.0	8.8	9.3	11.1	11.5	13.2	13.4	16.4	18.3	18.0	21.1	22.1	27.4
Av. monthly t°C	19.6			12.7			6.6			2.2			-3.9			3.2			9.7			12.7			17.6			22.8		
Av. for the period 1965/95	18.3			12.6			7.4			2.2			-0.4			2.2			6			12.2			17.2			20.9		

Table 2. Sum of rainfall (mm/m<sup>2</sup>) by ten days in the study period 2014-2017

Months	IX			X			XI			XII			I			II			III			IV			V			VI		
	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
Ten days	78.2	66.5	51	5	0.1	116	0.5	48.7	0.3	78.3	6.2	8.5	0.1	0.0	17.3	72.4	0.6	3.6	102.0	28.7	7.3	12.6	0.2	1.2	28.6	36.2	4.7	3.0	49.1	24.6
Monthly amounts	195.7			121.1			49.5			93.0			17.4			76.6			138.0			14.0			69.5			76.7		
2015/16	3.0	71.8	25.8	12.4	18.3	39.6	0	0	39.6	0	2.2	1.4	15.3	54.3	0	15.1	5.3	4.0	7.8	23.0	3.1	0.1	15.4	15.2	24.8	32.2	7.7	37.2	9.4	13.1
Monthly amounts	100.6			70.3			39.6			3.6			69.6			24.4			33.9			30.7			64.7			59.7		
2016/17	0.2	0.1	2.0	2.2	17.1	0.4	5.9	3.6	23.4	0.0	0.0	2.4	22.2	46.8	1.1	5.4	3.2	2.5	33.3	10.3	4.3	1.9	20.9	3.3	27.2	0.7	24.8	2.8	3.3	9.3
Monthly amounts	2.3			19.7			32.9			2.4			70.1			11.1			47.9			26.1			52.7			15.4		
Av. for the period 1965/95	65			47			35			36			40			48			44			39			32			36		

In 2017, during the tillering phase, higher values for plant height were reported in the

variant with self-fertilization with Agriorgan pellets - 14.80 cm (14% above the control).

Table 3. Height of plants by phenological phases and organic fertilization variants, *Triticum spelta* L. (2015-2017), cm

Year Variants	Phenological phases														
	Tillering					Stem elongation					Full ripeness				
	2015	2016	2017	Average	%	2015	2016	2017	Average	%	2015	2016	2017	Average	%
Control	-	17.47	12.97	15.22	100	-	45.97	48.87	47.42	100	-	83.48	92.97	88.22	100
Agriorgan pellets	11.93	16.03	14.80	14.25	93.6	56.10	43.57	48.30	49.32	104.0	81.87	83.30	96.20	87.12	98.7
Amalgerol	12.80	15.88	14.43	14.37	94.4	58.33	49.10	50.20	52.54	110.8	75.57	87.52	101.4	88.16	99.9
Lithovit	12.87	15.67	12.47	13.67	89.8	60.67	55.60	54.86	57.04	120.3	83.27	81.65	97.77	87.56	99.2
Baikal EM	11.63	15.37	14.30	13.77	90.5	54.93	51.07	53.23	53.08	111.9	84.35	68.03	99.60	83.99	95.2
Tryven	12.30	16.87	13.37	14.18	93.2	55.97	51.13	56.93	54.68	115.3	76.93	87.15	96.97	87.02	98.6

Consecutive release of nutrients from the organic fertilizer and their absorption by the plants in the soil coenosis promotes their fuller assimilation and good vegetative growth. Higher plants were also reported in the variants, treated with Amalgerol (11% above control) and Baikal EM (10% above control). In the stem elongation phase there is an increased effect on growth and higher plant height than already absorbed foliar fertilizers, and this effect is best expressed in the variants treated with Tryven- 16%, Lithovit- 12% and Baikal- 9% above control. The tendency for higher plants is maintained in full ripeness phase at the variants, treated with Amalgerol (9% above control) and Baikal (7% above control). It is very clearly stated that in all variants with applied foliar fertilizers there are higher values for plant height compared, to the control values. This proves that foliar vegetation nutrition has a positive and timely effect on plant nutrition. Average values for the study period show, that in the tillering phase, the heights of the plants from the different fertilization variants are close. This can be explained by the still weak reaction of plants to feeding with foliar fertilizers in the phenological phase itself. A more noticeable

effect is observed at variants, treated with Amalgerol and Tryven. In the stem elongation phase, higher values of the indicator are reported when treated with Lithovit, Tryven, Baikal EM and Amalgerol, respectively 20%, 15%, 12% and 10.8% above the control variant. There is a noticeable increase in plant height at phenological phase in the treated variants and it shows that the combined application of biofertilizers in the growing technology leads to better nutrition and better vegetative growth of the plants. In the phase of full ripeness it is noticed that the application of biofertilizers have a stimulating effect on the plants height at *Triticum spelta* and their usage is a reliable measure to support better nutrition and vegetative growth of plants to achieve higher grain yields.

**3. 'Soil respiration' in *Triticum spelta* L.** The results for 'soil respiration' after the first vegetative feeding in 2016 at *Triticum spelta* L., show that Amalgerol application, leads to highest soil activity on the 7<sup>th</sup> day and confirmed on the 14<sup>th</sup> day, which is preserved during the second reporting of the heading phase (Table 4).

Table 4. Data on 'Soil respiration' - ( $\mu\text{g CO}_2/\text{h/g}$ ) in *Triticum spelta* L. (2016)

Variants	7 <sup>th</sup> day	14 <sup>th</sup> day	21 <sup>st</sup> day
	Average $\pm$ St. dev.	Average $\pm$ St. dev.	Average $\pm$ St. dev.
	First reporting (phase stem elongation)		
Control	12.23 $\pm$ 0.208 <sup>a</sup>	12.00 $\pm$ 1.000 <sup>ab</sup>	7.13 $\pm$ 0.115 <sup>b</sup>
Agriorgan pellets	9.80 $\pm$ 0.173 <sup>c</sup>	10.67 $\pm$ 0.577 <sup>b</sup>	9.03 $\pm$ 1.266 <sup>a</sup>
Amalgerol	12.60 $\pm$ 0.100 <sup>a</sup>	12.33 $\pm$ 0.577 <sup>a</sup>	9.37 $\pm$ 0.321 <sup>a</sup>
Lithovit	10.90 $\pm$ 1.217 <sup>bc</sup>	11.67 $\pm$ 0.577 <sup>ab</sup>	9.13 $\pm$ 0.503 <sup>a</sup>
Baikal EM	10.37 $\pm$ 0.208 <sup>c</sup>	11.00 $\pm$ 1.000 <sup>ab</sup>	9.53 $\pm$ 0.058 <sup>a</sup>
Tryven	12.00 $\pm$ 0.985 <sup>ab</sup>	11.67 $\pm$ 0.577 <sup>ab</sup>	9.70 $\pm$ 0.265 <sup>a</sup>
	Second reporting (phase heading)		
Control	8.3 $\pm$ 0.723 <sup>b</sup>	8.4 $\pm$ 0.115 <sup>d</sup>	7.6 $\pm$ 0.404 <sup>b</sup>
Agriorgan pellets	8.0 $\pm$ 0.208 <sup>b</sup>	8.1 $\pm$ 0.173 <sup>d</sup>	8.1 $\pm$ 0.265 <sup>b</sup>
Amalgerol	10.2 $\pm$ 1.852 <sup>a</sup>	9.9 $\pm$ 0.115 <sup>a</sup>	9.3 $\pm$ 0.493 <sup>a</sup>
Lithovit	8.3 $\pm$ 0.115 <sup>b</sup>	9.4 $\pm$ 0.208 <sup>b</sup>	7.4 $\pm$ 1.159 <sup>b</sup>
Baikal EM	8.7 $\pm$ 0.416 <sup>ab</sup>	9.8 $\pm$ 0.289 <sup>a</sup>	9.2 $\pm$ 0.265 <sup>a</sup>
Tryven	8.9 $\pm$ 0.265 <sup>ab</sup>	8.97 $\pm$ 0.208 <sup>c</sup>	9.6 $\pm$ 0.404 <sup>a</sup>

\*Means followed by the same letter are not statistically different ( $P < 0.05$ ) by Duncan's multiple range test

This gives a reason to attribute this effect to the composition of the fertilizer and the increased microbial activity in the rhizosphere zone, which has an impact on the acceleration of assimilation processes, expressed in maximum height in plants during full ripeness phase - 87.52 cm.

It can be pointed out that at the first reporting (stem elongation) on the 14<sup>th</sup> day is observed an increase in soil activity at the variants, treated with Lithovit and Tryven, which has its impact on vegetative growth in the stem elongation, as plants treated with Lithovit having the largest height- 55.60 cm, followed by Tryven- 51.13 cm.

The activation of the rhizosphere microflora under the influence of Lithovit biofertilizer is associated with the rich in micronutrients composition of the fertilizer.

It is necessary to point out that in the first reporting (stem elongation) on the 21<sup>st</sup> day, in

addition to activity in Tryven- 9.70  $\mu\text{g CO}_2/\text{h/g}$ , there is a significant activity in the plants of the variant treated with Baikal EM- 9.53  $\mu\text{g CO}_2/\text{h/g}$ , which also affects the greater height of the plants in the stem elongation phase- 51.13 cm at Tryven treated variant, and 51.07 cm when Baikal EM is used.

In the days before sampling, the environmental conditions were reported as favorable, improving the microbial activity, namely - precipitation 14.3 mm, soil temperature (at 10 cm depth) - 17.7 °C, air temperature - 15.9°C and relative humidity - 60% (Table 1).

The results of soil microbial activity in 2017 after the first feeding show that on the 7<sup>th</sup> day in all variants with foliar biofertilizers there is a proven higher soil activity in plants, and it is best expressed in the variant with biofertilizer Amalgerol (Table 5).

Table 5. Data on 'Soil respiration' - ( $\mu\text{g CO}_2/\text{h/g}$ ) in *Triticum spelta* L. (2017)

Variants	7 <sup>th</sup> day	14 <sup>th</sup> day	21 <sup>st</sup> day
	Average $\pm$ St. dev.	Average $\pm$ St. dev.	Average $\pm$ St. dev.
	First reporting (phase stem elongation)		
Control	9.10 $\pm$ 0.376 <sup>b</sup>	9.00 $\pm$ 0.608 <sup>c</sup>	8.1 $\pm$ 0.153 <sup>d</sup>
Agriorgan pellets	9.30 $\pm$ 0.095 <sup>b</sup>	9.03 $\pm$ 0.569 <sup>c</sup>	8.2 $\pm$ 0.100 <sup>d</sup>
Amalgerol	11.9 $\pm$ 0.648 <sup>a</sup>	11.89 $\pm$ 0.081 <sup>a</sup>	11.4 $\pm$ 0.100 <sup>a</sup>
Lithovit	11.30 $\pm$ 0.458 <sup>a</sup>	11.50 $\pm$ 0.520 <sup>ab</sup>	9.6 $\pm$ 0.100 <sup>c</sup>
Baikal EM	11.7 $\pm$ 0.060 <sup>a</sup>	11.70 $\pm$ 0.105 <sup>a</sup>	10 $\pm$ 0.265 <sup>b</sup>
Tryven	11.5 $\pm$ 0.202 <sup>a</sup>	10.80 $\pm$ 0.173 <sup>b</sup>	9.6 $\pm$ 0.100 <sup>c</sup>
	Second reporting (phase heading)		
Control	9.0 $\pm$ 0.361 <sup>d</sup>	9.1 $\pm$ 0.058 <sup>c</sup>	8.0 $\pm$ 0.153 <sup>d</sup>
Agriorgan pellets	9.2 $\pm$ 0.115 <sup>d</sup>	9.2 $\pm$ 0.208 <sup>c</sup>	8.2 $\pm$ 0.252 <sup>cd</sup>
Amalgerol	11.1 $\pm$ 0.252 <sup>a</sup>	10.9 $\pm$ 0.252 <sup>a</sup>	10.6 $\pm$ 0.265 <sup>a</sup>
Lithovit	9.8 $\pm$ 0.513 <sup>c</sup>	10.1 $\pm$ 0.208 <sup>b</sup>	8.5 $\pm$ 0.100 <sup>c</sup>
Baikal EM	10.4 $\pm$ 0.115 <sup>b</sup>	10.7 $\pm$ 0.058 <sup>a</sup>	9.6 $\pm$ 0.100 <sup>b</sup>
Tryven	10.7 $\pm$ 0.153 <sup>ab</sup>	10.83 $\pm$ 0.058 <sup>a</sup>	10.9 $\pm$ 0.265 <sup>a</sup>

\*Means followed by the same letter are not statistically different ( $P < 0.05$ ) by Duncan's multiple range test

This tendency for higher activity when treated with Amalgerol is maintained on the 14<sup>th</sup> and 21<sup>st</sup> day after treatment, which leads to greater vegetative growth by *Tr. spelta* in the phase of full ripeness, where the plants reach the highest height of 101.4 cm (Table 3). A similar consistent effect is observed when Baikal EM is applied, which leads to values for the greater height in the phase of full ripeness - 99.60 cm, compared to the control. In the second reporting at heading phase, the same trend is observed for overall positive impact of feeding with the studied biofertilizers, as again the variant treated with Amalgerol comes to the fore, in which proven higher values of soil respiration from 7<sup>th</sup> to 21<sup>st</sup> are reported and the day after treatment of the plants. A similar trend is observed at the variant treated with Tryven, followed by the variant treated with Baikal EM.

## CONCLUSIONS

Higher plant height as a result of the applied biofertilizers was reported at tillering phase 14 days after treatment with Lithovit, Tryven, Baikal EM and Amalgerol, where the increase of the indicator compared to the control is by

20%, 15%, 12% and 10.8%. The combined application of biofertilizers in the growing technology leads to better nutrition and better vegetative growth of plants.

Over the years proven higher values of soil respiration 7 days after treatment were reported in the variants treated with Amalgerol, Baikal EM and Tryven. Greater soil microbial activity in the studied biofertilizers was observed on the 14<sup>th</sup> day after treatment. Higher values are reported for all variants of combined fertilization (foliar fertilization on basic fertilization with Agriorgan pellet) compared to unfertilized control and self-fertilization with Agriorgan pellet, which is statistically proven. On the 21<sup>st</sup> day after biological fertilizers application, a slight decrease in the values of the soil microbial activity is observed in all treated variants and it starts to approach the value of the control.

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# MISCELLANEOUS



## CONTRIBUTION TO THE EVALUATION OF *Citrullus colocynthis* (L.) SCHRAD AS POTENTIAL BIODIESEL FEEDSTOCK: SEED YIELD, OIL CONTENT AND YIELD COMPONENTS

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### Abstract

*Citrullus colocynthis* is a perennial herbaceous species in the Cucurbitaceae family widely distributed in the Sahara Arabian desert in Africa and the Mediterranean region. This plant has a natural adaptation to drought and several studies have reported that this plant has a great potential for adaptation to drought with a tolerance to water deficit. *C. colocynthis* has been garnering interest in recent times as a potential biodiesel feed stockcrop due to its high seed oil content. The variability of seed and oil yields were investigated for 12 accessions collected in different localities in Morocco and analyze during two consecutive trials. The measured traits are: seed number (SN), seed weight (SW), weight of 100 seeds (W100), seed oil content (OC), seeds yield (SY) and oil yield (OY). The seed oil content ranging between 17 and 24% of seed weight. The extrapolated seed yield among the accessions ranged from 156 to 816 kg/ha, with an overall mean of 413 kg/ha. The oil yield varied between 35 to 172 kg/ha. The results of anova (GLM) show significant and very highly significant differences between the accessions according to the characters measured.

**Key words:** *Citrullus colocynthis*, seed oil content, seeds yield, oil yield, Morocco.

### INTRODUCTION

*Citrullus colocynthis* (L.) Schrad (2n = 22), closely related to domesticated watermelon (*Citrullus lanatus* var. *lanatus*) (Chomicki & Renner, 2015), is a very drought-tolerant perennial herbaceous species in the Cucurbitaceae family.

It is a wild native plant growing in arid areas, widely distributed in the Sahara-Arabian desert in Africa and the Mediterranean region (Si et al., 2010; Wang et al., 2014). In recent years, the development of new oil seed crops that can be used as alternatives to conventional plants has generated a lot of interest and *C. colocynthis* is one of the plants that is able to adapt to arid conditions.

This plant has a natural adaptation to drought and several studies have reported that this plant has a great potential for adaptation to drought with a tolerance to water deficit (Hassan et al., 2012; El Madidi et al., 2017; Verma et al., 2017). *C. colocynthis* being an exceptionally hardy plant with a potential for use as biodiesel feedstock (Giwa et al., 2010; Bello and Makanju, 2011). The plant was also shown to be rich in nutritional value with high protein

contents and important minerals as well as edible quality of seed oil (Hussain et al., 2014).

### MATERIALS AND METHODS

12 accessions of *C. colocynthis* collected from different regions from Morocco (Figure 1). The trial was carried out during 2 growing seasons 2016-17 and 2017-18 at the experimental farm of Sidi Bibi (30° 15' 00" N, 9° 30' 36" W), following a randomized block experimental design with 3 replicates for each trial. The measured traits are: seed number per plant (SN), seed weight per plant (SW), weight of 100 seeds per plant (W100), oil content of seed (OC), Seed yield (kg/ha) and Oil yield (kg/ha). The lipid components of the *C. colocynthis* seeds were subsequently extracted with n-hexane using a Soxhlet apparatus at 45-50°C for 6-8 h until the extraction was completed. The crushed seeds (approximately 400 g) of *Citrullus colocynthis* were placed in a Soxhlet apparatus and then extracted with 300 mL n-hexane for 7 hours on a water bath. Lipid fraction was extracted in a Soxhlet apparatus for 16 h at 60°C. The solvent was evaporated, and the lipid fraction residues were weighed.

Data were analyzed by the GLM procedure of SAS with the following statistical model:

$$Y_{ijk} = m + A_i + B_j + AB_{ij} + C_k + E_{ijke}$$

Where:  $A_i$ : effects of accessions,  $B_j$ : effects of years,  $AB_{ij}$ : effects of interaction accessions years,  $C_k$ : effects of blocs and  $E_{ijke}$ : residual error. Statistics analysis was carried out using computer software SAS version 9.3 (SAS Institute Inc., 2010).

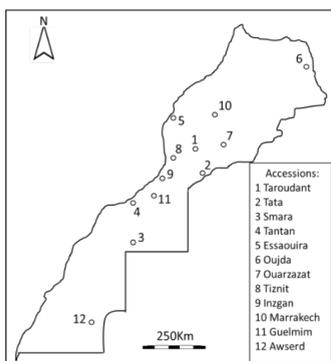


Figure 1. Origin of accessions of *Citrullus colocynthis* (L.) Schrad collected in several localities in Morocco

## RESULTS AND DISCUSSIONS

Analysis of the data shows a high degree of variability with the coefficient of variation (CV) ranged from 7.65 % to 67.34 %. The seeds weight per plant has the highest CV and the values are ranged from 4.09 to 198.40 grams, while for the seeds number the values vary from 121 to 1467 seeds per plant. The seed yields ranging between 155.78 and 815.76 kg per hectare and the oil yield varies between 35.27 and 171.71 kg/hectare while the seed oil content (OC) shows the lowest CV and the minimum and maximum are respectively 17.1 and 24.3 % (Table 1).

Table 1. Descriptive statistics for the measured characters

	m	Min	Max	SD	CV(%)	SE
SN/P	770.08	121.00	1467.00	510.05	66.23	19.01
SW/P	42.66	4.09	198.40	28.73	67.34	1.07
W <sub>100</sub>	5.562	3.01	9.63	0.772	13.88	0.016
SY(Kg/ha)	419.87	155.78	815.76	159.94	39.09	18.85
OC (%)	21.41	17.10	24.29	1.64	7.65	0.19
OY(Kg/ha)	89.84	35.27	171.70	34.99	38.96	4.12

SN/P: seed number per plant, SW/P: seed weight per plant (g), W<sub>100</sub>: weight of 100 seeds SY: Seeds Yield (kg/ha), OY: Oil Yield (kg/ha) and OC: oil content, m: mean, Min: Minimum, Max: Maximum, SD: Standard Deviation, CV: Coefficient of Variation and SE: Standard error.

Table 2. Results of GLM Anova (Fisher-Snedecor values and significant levels)

SV	SN	SW	W <sub>100</sub>	OC	SY	OY
Accessions	12.55 ***	10.74 ***	13.50 ***	9.52 ***	12.20 ***	6.67 ***
Years	33.82 ***	15.93 ***	27.99 ***	13.55 **	6.24 **	8.71 **
Acc x Y	1.50	0.95	3.16 *	2.61 *	3.29 *	0.66
Blocs	2.31	2.94 *	2.25	1.24	1.40	1.75

\*\*\*, \*\*, \*: was significant at 0.001, 0.01 and 0.05 level respectively.

The analysis of variance (ANOVA) indicated high statistically significant differences among the 12 accessions for all the characters analyzed (Table 2). Highly significant differences are also observed between the trials of the two successive years. The interaction accessions x years was significant for SY, W<sub>100</sub> and OC.

Table 3. Mean values and classification into homogeneous groups of the 12 accessions of *Citrullus colocynthis* (L.) Schrad

	SN	SW	SY	OC	OY	W <sub>100</sub>
A1	723.8 bc	40.51 bcd	405.09 abc	21.02 bc	84.88 abc	5.59 cd
A2	1084.28 ef	58.67 fj	586.67 de	21.04 bc	123.11 de	5.45 bc
A3	643.25 abc	34.57 abc	346.91 ab	23.19 e	80.43 ab	5.46 bc
A4	517.32 a	29.36 a	289.05 a	21.97 cde	63.43 ab	5.72 ce
A5	918.33 de	49.24 def	375.69 ab	21.09 bc	78.2 ab	5.36 b
A6	774.27 cd	44.4 cde	451.48 bcd	19.25 a	87.56 abc	5.84 e
A7	650.72 abc	36.91 abc	369.09 ab	20.02 ab	73.97 ab	5.69 ce
A8	566.48 ab	30.43 ab	304.28 ab	21.78 cd	66 ab	5.24 a
A9	814.92 cd	42.25 cd	418.76 abc	22.93 cde	96.25 bcd	5.18 a
A10	539.77 ab	30.06 ab	306.89 ab	20.12 ab	61.21 a	5.78 ce
A11	1100.45 f	62.4 j	640.69 e	22.96 de	146.97 e	5.62 cd
A12	905.84 d	52.99 fj	543.90 cde	21.53 c	116.11 cde	5.82 ce

Means within columns with different superscript are significantly different ( $p < 0.01$ ) using the Ducan New Multiple Range Test (DMRT).

The mean values of the variables measured and the distribution of the 12 accessions into homogeneous groups are given in Table 3. For seed yield, average values were grouped into 5 homogeneous groups and ranged from 289.05 kg/ha (accession 4) to 640.7 kg/ha (accession

11). For oil yield, the average values were arranged into 5 homogeneous groups and varied between 61.2 kg/ha (accession 10) and 146.97 kg/ha (accession 11). 7 homogeneous groups are observed for the seeds weight per plant (SW) and the lowest value is recorded for A4 while the highest value is recorded for A11. For the seeds number per plant (SN), the means values vary from 517 to 1100 seeds per plant which are measured respectively in A4 and A11.

Table 4. Ranking order for different characters of the 12 accessions

	SN	SW	SY	OC	OY	W <sub>100</sub>	AR
A1	7	7	6	8	6	7	6.83
A2	2	2	2	9	2	9	4.33
A3	9	9	8	1	7	8	7.00
A4	12	12	11	4	11	4	9.00
A5	3	4	7	7	8	10	6.50
A6	6	5	4	12	5	1	5.50
A7	8	8	12	11	9	5	8.83
A8	10	10	10	5	10	5	8.33
A9	5	6	5	3	4	12	5.83
A10	11	11	9	10	12	3	9.33
A11	1	1	1	2	1	6	2.00
A12	4	3	3	8	3	2	3.83

AR : Average Rank

The order of classification of the 12 accessions is presented in Table 4 which shows that the classification of the accessions according to the variable analysed. The lowest averages rank values are observed for A11, A12 and A2 while the highest averages values are attributed to A10, A4, A7 and A8. Accession A11 recorded the lowest average ranking, it is the first in terms of SN, SW, SY and OY, and second for OC. The highest average rank value is observed for A10, it is the 12th for OY, 11th for SN and SW, 10th for OC and 9th for SY.

The seed yield values obtained in this study under semi-arid conditions without any fertilizer and under conditions of limited irrigation, remain relatively low compared to those potentially obtained under optimal growing conditions. The seed yield values obtained in this study under semi-arid conditions without any fertilizer and under conditions of limited irrigation, remain relatively low compared to those potentially obtained under optimal growing conditions. The authors of a study conducted on the production of *Citrullus colocynthis*, reported

that production can reach 4400 kg of seeds and 1000 to 1175 L/ha of colocynth vegetable oil in rainy conditions (Mertia & Gupta, 1994). With plant density of four plants per m<sup>2</sup>, the extrapolated annual seed yield among the accessions of *Citrullus colocynthis* ranged from 0.47 to 14.95 tons/ha, with an overall mean of 5.17 tons/ha (Menon et al., 2016). In the desert in Pakistan, some accessions of *C. colocynthis* developed an extensive root system despite of receiving only receiving only 35-40 mm of rainfall, can be produced as much as 1-1.5 t of seed/ha, but as much as 40-fold more if rainfall is higher (Mahajan et al., 2013).

This plant has the potential to be used for grain production for oil extraction, especially under difficult conditions on arid and semi-arid lands and the exploitation of the natural variability could be used for the selection of accessions that have good oil yields in limited growing conditions (El Madidi & Benmoumou, 2020). To reduce their energy dependence and decrease their carbon emissions, many countries have encouraged the use of biofuels, but competition between edible oil and fuel needs can lead to higher prices for essential foodstuffs and global disproportion in fuel consumption and for the food industry and market demands. Knowing that the use of all edible oils for the production of biodiesel will not be enough to meet the global demand for biofuel (Kumar & Sharma, 2011; Taufiq-Yap et al., 2011). Therefore, there is a need for an inexpensive, inedible raw material such as *Citrullus colocynthis* oil for the production of biodiesel. This plant being desert shrub and semi-xerophytic in nature, require less water and can tolerate saline as well as alkaline soils and that grows in many countries in the arid or semi-arid lands and its seeds are rich in oil, which can encourage their sustainable agriculture to produce economical biodiesel at a competitive price (Elsheikh et al., 2014).

## CONCLUSIONS

The results obtained in this study show that this plant has good potential to be used for the seeds production for oil extraction, especially in difficult conditions in arid, semi-arid and wastelands. The great variability in seeds and oil yield suggests that better yields can be

obtained by analyzing the best growing conditions and selecting the best accessions for a yield with good tolerance to different abiotics stress.

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## STUDY ON THE USE OF THE LAND SCAN TO DETERMINE THE SOIL VOLUME DISLOCATED BY EROSION OF DEPTH ON THE PRACTICE OF SUSTAINABLE AGRICULTURE IN AGRITOURISTIC FARMS

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### Abstract

*The paper presents a continuation of the study conducted in the paper "Study on the use of land scanning in soil erosion inventory works for sustainable agriculture in agritourism farms" because we found that it is very important to determine the land areas affected by the phenomenon of deep erosion, to present a modern, accurate and fast method of determining the volume of dislocated soil. It is known that a very large amount of soil is washed from that surface and with it the organic matter, which takes hundreds of years to recover, at its initial value. In order to support landowners who own such areas affected by erosion, we thought of presenting a precise and very effective method of monitoring the volume of soil displaced. The method that was imposed from the study is to determine the volume by terrestrial laser scanning, which also has a number of advantages, compared to the classical methods used so far. The terrestrial laser scanning through the large number of data collected from the field offers us the possibility to determine with great ease the total cubage of the studied negative relief form, but also gives the possibility to develop thematic digital 3D maps, which farmers can use in practicing sustainable agriculture, based on precision agriculture.*

**Key words:** *coordinates, cubage, land scanning, point cloud, sustainable agriculture.*

### INTRODUCTION

As is well known, agriculture plays an important role in the conservation of natural resources and cultural landscapes and is a prerequisite for other human activities in rural areas. Over the centuries, agriculture has contributed to the creation and preservation of a wide variety of landscapes and habitats (Călina & Călina, 2019). However, agricultural practices can also have negative effects on the environment, effects that are most visible in agritourism farms, as they are located in more remote places, with beautiful landscape and fragmented relief (Adamov et al., 2020).

The European Union's rural development policy provides funding for a wide range of measures that Member States or regions use to support the sustainable development of rural areas (Călina & Călina, 2019). The agri-environment measures within the axes also aim at soil degradation on agricultural land, stimulating farmers to protect, maintain and improve the quality of the environment on the land of their farm (Iagăru et al., 2016).

The phenomenon of soil erosion by water, according to the data of the National Research

and Development Institute for Pedology, Agrochemistry and Environmental Protection (ICPA) affects in Romania approx. 6.3 million ha, of which 2.1 million ha of arable land, to which is added another 0.378 million ha (0.273 million ha of arable land) subject to wind erosion (Răduțoiu & Stan, 2013). Of the known forms of degradation, the most severe and widespread is water erosion. As the erosion processes advance, the soil loses its energetic potential and its ecological functions, approaching the parent rock by its properties. Thus, erosion occurs the "counterrevolution of soils" (Burghilă et al., 2016).

In this paper, the achievement of the proposed objective is possible through the use of the geoinformation system, which allows the operative and reasoned approach to the problems of inventory, analysis, planning and design. Thanks to it, premises are created for the elaboration and implementation of measures to combat soil degradation at a new quality level, aimed at maintaining and improving soil fertility. Within this system the essential component is represented by the terrestrial laser scanning.

The advantage of laser scanning is that it can record a large number of points, at a high accuracy, in a relatively short period of time. It's like taking a picture with depth information. The combination of the rotating optical elements and the moving mechanisms of the instrument offers the reflected laser beam the possibility to create a uniform network (grid). Through it the geometry of a structure can be measured completely automatically (more or less). The result of the measurements is represented by a (considerable) set of points called in the literature point cloud (Barazzetti et al., 2010).

## MATERIALS AND METHODS

In solving the topic, the same method was used as in the study "Study on the use of land scanning in soil erosion inventory works for sustainable agriculture in agritourism farms" which involved the use of Terrestrial Laser Scanning (SLT) technology, which tends to "revolutionize" measurement techniques in Topography and Engineering Topography (Calinovici & Călina, 2008). The use of this technology allowed the rapid and precise determination of the surface and cubage of a formation that appeared on an agricultural surface, following deep erosion. As with any measurement process encountered in the technique of topographic measurements and in this case the planning or preliminary design is an extremely important step, decisive in obtaining the results, respectively the information needed to describe the object to be scanned (Kolbe et al., 2011).

The design stage is indispensable for the measurement process and due to the fact that in this stage the shape and size of the object are balanced, its position in the environment and last but not least, the requirements of the beneficiary regarding the accuracy to be obtained in object representation at the end (Li et al., 2009).

After a rigorous design, it was concluded that the following phases must be completed: - definition of the area to be scanned and preliminary investigations; - determining the resolution and accuracy required for the points that make up the point clouds, depending on the beneficiary's sorting; - selecting the type of

laser scanner to be used, depending on the specifics of the work we intend to perform; - designing the optimal positions of the station points for scanning, starting from the premises of providing the necessary coverage to ensure accuracy and the need to scan the entire object; - the choice of the type of targets that will be used in the georeferencing registration operations and of the positions in which they will be located, in such a way as to ensure the premises of an optimal geometric configuration for georeferencing; - estimating the volume of data that will be acquired during the scanning process (Remondino et al., 2010).

In the case of the relief form studied, it was considered that the use of terrestrial laser scanning is the most optimal due to the following reasons: - very complex surface structure; - presentation of the final 3D product; - measuring the surface instead of measuring the individual points; - the recorded data can be used by multidisciplinary teams, for different purposes; - archiving data without having a priori knowledge, regarding their future use (Mihai et al., 2015).

### Stage I. *Analysis of the area to be scanned*

Retrieving as much information as possible about the object to be scanned can provide information about the complexity and time required for such an operation. Field information, reports, existing maps, photographs or video images of the location of the object to be scanned can help a lot in determining possible risks when scanning the object (Păunescu et al., 2020). However, it is also very important to analyze the area, the surroundings of the respective location. Possible obstructions determine the choice of station point positions. Possible time constraints are also decisive in choosing the methods and timing of the scan. Indirectly, the positions of the station points determine the minimum and maximum distances that the scanner could record.

### Stage II. *Determining the optimal positions for scanning*

Once the site documentation has been analyzed and laser scanning has been chosen as the most effective recording technique, the scanning positions and those in which the aiming targets will be placed must be designed (planned). The choice of the optimal positions of the station

points must guarantee maximum coverage and accuracy but also a minimum number of station points.

When designing the optimal positions of the station points, the following basic rules must be observed: - the positions are chosen that offer a good (wide) coverage of the scanning area, without obstructions on the line of sight, which could produce the shading effect; - it is checked whether the distance limits are met, in order to increase the accuracy; - decrease in the number of scanning stations; - choosing scanning positions in places that ensure comfortable measuring conditions, free from vibrations and the influence of wind; - ensuring a convenient height of the device and ensuring visibility to natural and artificial targets.

Of particular importance in the measurement design phase, in addition to the optimal scanning positions, is the choice of target types, their position and / or geometric configuration. An important remark regarding the use of aiming targets is that they, placed in position, must have a large opening in all three directions of the axes (X, Y, Z).

#### Stage III. *Data management*

Given the very large amount of data that is collected during the scanning process, it is very important to scan the data so that it is ensured throughout a working day. The positioning accuracy of the image points, defined in the reference system of the station by spatial coordinates X, Y, Z is accredited at  $\pm 6\text{mm} / 50\text{m}$ , given that at this distance the laser spot maintains its point diameter of 6 mm. Currently, there is no standard procedure for planning the terrestrial laser scanning session (Doneus et al., 2005).

Based on the records made, the so-called "point cloud" is obtained, which is a collection of points, defined as a position by the coordinates X, Y, Z in a common reference system, which reveals to the observer information on the shape, position, and the spatial distribution of an object or group of objects (Călina et al., 2020). It may contain additional information, such as intensity. It can be concluded that the point cloud contains two types of information: - metrics, which describe the geometry of the object and its spatial relationships with the environment; - thematic, which are used to describe the surface properties of the scanned

objects and to estimate the confidence given to the acquired data.

## RESULTS AND DISCUSSIONS

The idea of conducting this study arose with previous research in the paper "Study on the use of land scanning in soil erosion inventory works for sustainable agriculture in agritourism farms" from which we found that estimating soil volumes is very important for several applications such as: soil erosion studies, estimation of ore removed from a surface in the mine, evaluation of construction land, etc. Due to the way of taking data remotely, laser scanners have proven to be optimal for this type of work, being able to easily delimit the volume of interest and provide a large and complex amount of data and information (Călina et al., 2018).

This method was chosen because it is known from the literature that many practical comparative applications have been made between laser scanning and conventional methods of data collection, to assess the accuracy and development of procedures to optimize the use of the method in embankment calculations. Based on these studies, it has been established that terrestrial scanners reach the desired level of accuracy in the shortest possible time and quickly provide information on the initial stage of the work required in the design phase or deviations from the project (Chiabrandu et al., 2009).

Field operations began with defining the area to be scanned and conducting preliminary investigations, in order to better document and plan the project. This was followed by another important operation in determining the resolution and accuracy required for the points that make up the point clouds, in accordance with the requirements of the beneficiary. Subsequently, the optimal positions of the terrestrial laser scan were established for a proper coverage of the studied area. At the same time, the time required to take over the data was estimated, given that it is a location that is constantly changing and of great complexity.

Further, in order to achieve the purpose of the work of estimating the volume of eroded soil, related to the initial land, the absolute

coordinates of the points in the local system were determined, because the determined area is the same as the one determined in the absolute system. In order to connect the measurements performed from several stations, it was necessary to place targets, whose position was established using classical

measuring means, landmarks that were the basis for "linking" the scanning and georeferencing stations (Rosca et al., 2020). Following the scanning of the studied surface, the georeferenced point cloud from the two scanning stations was obtained (Figure 1).

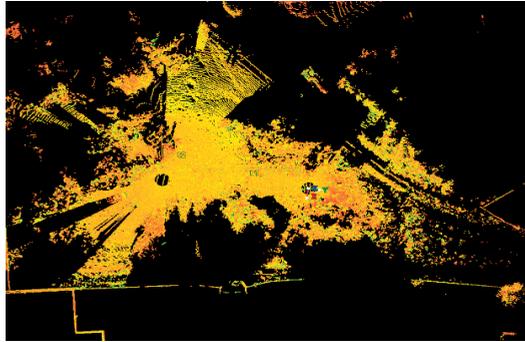


Figure 1. Recording of 2 scans: the point cloud in station 1, referenced with the one in station 2

### Calculation of point coordinates in the local system

We worked in the local reference system, this explains the negative coordinates of the points. This way of working is widely used in engineering works, because this way, the eventual tensions in the absolute coordinate system are not transmitted, which leads to

obtaining more precise coordinates. It was decided to work in this system because the purpose of the work was to determine the volume of the entire form of relief, and this is done regardless of the reference system chosen. It must be specified that due to the very large volume of points it was chosen to present only a small part of them, in facsimile (Table 1).

Table 1. Scan point coordinates

Point no.	Point coordinates (m)			Point no.	Point coordinates (m)			Point no.	Point coordinates (m)		
	X	Y	Z		X	Y	Z		X	Y	Z
1	-60.0518	-46.0209	-2.5567	83	-60.1324	-46.0257	-2.5631	165	-60.030	-46.1105	-2.4730
2	-60.0721	-46.0165	-2.5596	84	-60.1395	-46.0352	-2.5656	166	-60.0410	-46.1086	-2.4705
3	-60.0956	-46.0115	-2.5631	85	-60.1027	-46.0537	-2.5624	167	-60.0453	-46.0971	-2.4736
4	-60.1063	-46.0092	-2.5598	86	-60.1165	-46.0509	-2.5606	168	-60.0559	-46.1058	-2.4703
5	-60.1179	-45.9957	-2.5667	87	-60.1324	-46.0477	-2.5610	169	-60.0340	-46.1205	-2.4738
....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
35	-60.0483	-46.1180	-2.5455	117	-60.1371	-46.0466	-2.5056	199	-60.1346	-46.1019	-2.4749
36	-60.0867	-46.1109	-2.5577	118	-60.1335	-46.0584	-2.5027	200	-60.1847	-46.1259	-2.4725
37	-60.0670	-46.0931	-2.5571	119	-60.1776	-46.0273	-2.51059	201	-60.1799	-46.0935	-2.4730
38	-60.0410	-46.1300	-2.5495	120	-60.1537	-46.0322	-2.5068	202	-60.1793	-46.0825	-2.4736
39	-60.0411	-46.1405	-2.5399	121	-60.2021	-46.0335	-2.4963	203	-60.1792	-46.0826	-2.4646
40	-60.0542	-46.1276	-2.5476	122	-60.1496	-46.0662	-2.5018	204	-60.1899	-46.0917	-2.4607
....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
78	-60.0776	-46.0262	-2.5527	160	-60.0185	-46.1444	-2.4727	242	-60.5955	-45.7904	-2.5545
79	-60.1203	-46.0392	-2.5636	161	-60.0310	-46.1422	-2.4704	243	-60.5998	-45.7766	-2.5487
80	-60.1161	-46.0181	-2.5637	162	-60.0461	-46.1502	-2.4696	244	-60.5946	-45.8033	-2.5529
81	-60.1182	-46.0286	-2.5637	163	-60.0386	-46.1196	-2.4686	245	-60.6141	-45.7858	-2.5538
82	-60.1186	-46.0176	-2.5572	164	-60.0336	-46.0993	-2.4754	246	-60.6156	-45.7727	-2.5465
								247	-60.6391	-45.7927	-2.5634

**Representation of existing level curves in the field:** Based on the obtained point cloud and the coordinates of the points in the local

system, it was possible to perform a precise and fast representation of the level curves (Figures 2, 3 and 4).

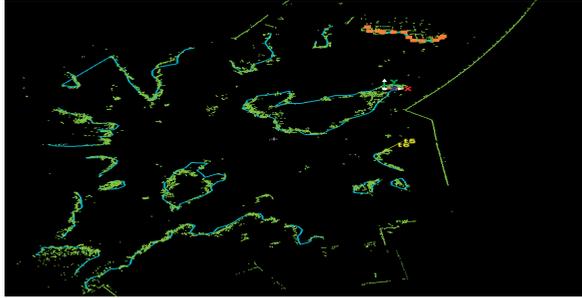


Figure 2. Point cloud at elevation  $h = 1.5$  m

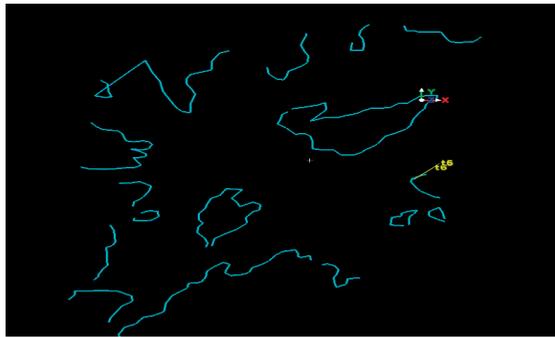


Figure 3. The level curves corresponding to the elevation  $h = 1.5$  m drawn from the point cloud

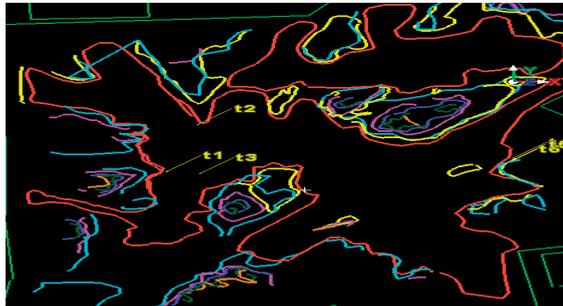


Figure 4. Level curves for relief shape; Red.  $h = 0.00$  m; yellow.  $h = 0.95$  m; blue.  $h = 1.5$  m; purple.  $h = 1.6$  m; navy blue.  $h = 2.4$  m; dark green.  $h = 2.88$  m; orange.  $h = 3.4$  m

### Calculation of the volume of eroded soil

Due to the fact that the relief form consists of several valleys, in order to arrive at the total calculation of the cubage, it was necessary to first calculate the cubage of each separate valley wire. In order to achieve this, each valley was divided into elementary geometric shapes (cylinders). Thus, elementary cylinders and the base areas of these valleys resulted, the

data being processed in the Cyclone program (Vosselman et al., 2010). Having the areas of the bases of the elementary cylinders in each valley and the height between them, the volume of the cubage of each valley was calculated, and finally that of the negative relief form, appeared on the agricultural land of the agritourism farm on which this study was conducted.

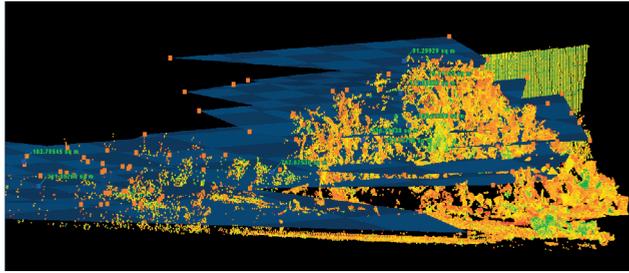


Figure 5. Base area values of the 10 elementary cylinders (image superimposed on the point cloud)

Point cloud processing is a difficult operation and requires a lot of attention (Sala et al., 2020). Using the values of the base areas of the 10

elementary cylinders in Figure 5 and the height between them, the volume of land displaced for the first valley wire was calculated (Table 2).

Table 2. Volume calculation for the first valley wire

Plan number	Surface, (m <sup>2</sup> )	Height, (m)	Volume, (m <sup>3</sup> )
1	103.795	0.5	51.8975
2	179.087	0.6	107.4522
3	241.092	0.35	84.3822
4	762.625	0.5	381.3125
5	313.227	0.35	109.62945
6	236.31	0.6	141.786
7	152.63	1	152.63
8	117.026	0.37	43.29962
9	75.711	0.83	62.84013
10	91.299	0.5	45.6495
<b>Total</b>			<b>1180.8791</b>

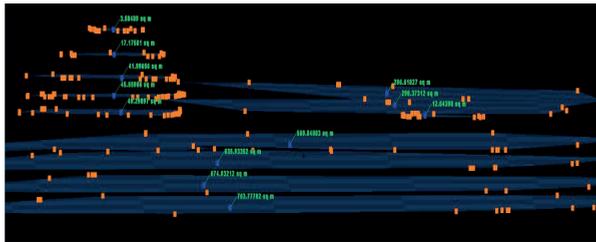


Figure 6. Values of the base areas of the elementary cylinders (Valley 2)

Using the values of the base areas of the 12 elementary cylinders in Figure 6 and the height

between them, the volume of displaced earth for the valley wire 2 was calculated (Table 3).

Table 3. Volume calculation for valley wire 2

Plan number	Surface (m <sup>2</sup> )	Height (m)	Volume (m <sup>3</sup> )
1	3.5	1	3.5
2	17.17	1	17.17
3	41.99	1	41.99
4	45.66	1	45.66
5	49.3	1	49.3
6	296.62	1	296.62
7	298.37	1	298.37
8	12.044	1	12.044
9	569.84	1	569.84
10	635.93	1	635.93
11	674.93	1	674.93
12	763.77	1	763.77
<b>Total</b>			<b>3409.124</b>

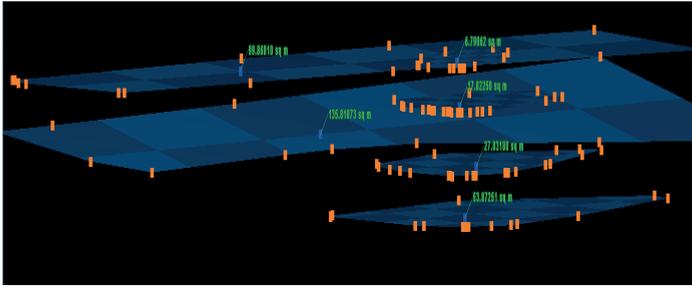


Figure 7. Values of the base areas of the elementary cylinders (Valley 3)

Using the values of the base areas of the 6 elementary cylinders in Figure 7 and the height between them, the volume of land displaced for the valley wire 3 was calculated (Table 4).

Table 4. Volume calculation for valley wire 3

Plan number	Surface (m <sup>2</sup> )	Height (m)	Volume (m <sup>3</sup> )
1	99.86	1	99.86
2	135.81	1	135.81
3	6.79	1	6.79
4	17.02	1	17.02
5	27.03	1	27.03
6	53.87	1	53.87
<b>Total</b>			<b>340.38</b>

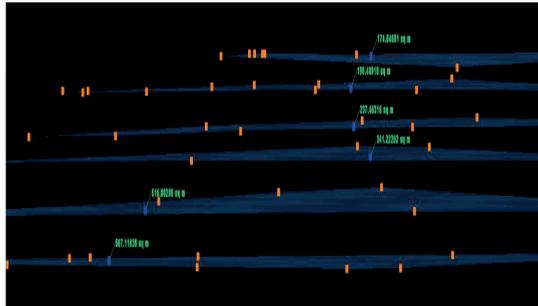


Figure 8. Values of the base areas of the elementary cylinders (Valley 4)

Using the values of the base areas of the 6 elementary cylinders in Figure 8 and the height between them, the volume of displaced earth for the valley wire 4 was calculated (Table 5).

Table 5. Volume calculation for valley wire 4

Plan number	Surface (m <sup>2</sup> )	Height (m)	Volume (m <sup>3</sup> )
1	174.04	1	174.04
2	199.49	1	199.49
3	237.46	1	237.46
4	341.22	1	341.22
5	516.08	1	516.08
6	587.11	1	587.11
<b>Total</b>			<b>2055.4</b>

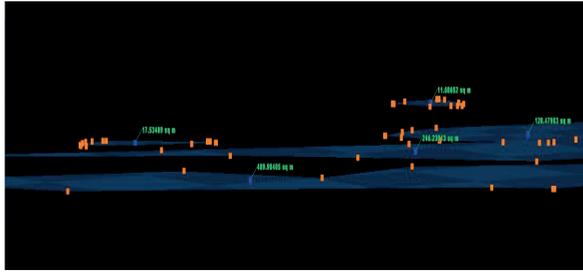


Figure 9. Values of the base areas of the elementary cylinders (Valley 5)

Using the values of the base areas of the 5 elementary cylinders in Figure 9 and the height between them, the volume of land displaced for the valley wire 5 was calculated (Table 6).

Table 6. Volume calculation for valley wire 5

Plan number	Surface (m <sup>2</sup> )	Height (m)	Volume (m <sup>3</sup> )
1	11.69	1	11.69
2	17.53	1	17.53
3	128.48	1	128.48
4	246.23	1	246.23
5	409.9	1	409.9
<b>Total</b>			<b>813.83</b>

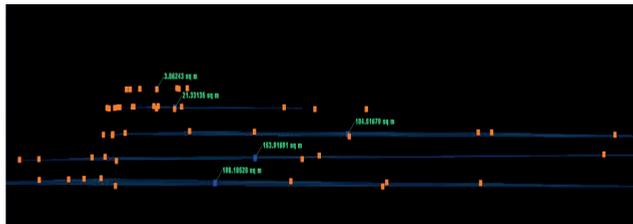


Figure 10. Values of the base areas of the elementary cylinders (Valley 6)

Using the values of the base areas of the 5 elementary cylinders in Figure 10 and the height between them, the volume of displaced earth for the valley wire 6 was calculated (Table 7).

Table 7. Volume calculation for valley wire 6

Plan number	Surface (m <sup>2</sup> )	Height (m)	Volume (m <sup>3</sup> )
1	3.86	1	3.86
2	21.33	1	21.33
3	104.51	1	104.51
4	153.91	1	153.91
5	198.18	1	198.18
<b>Total</b>			<b>481.79</b>

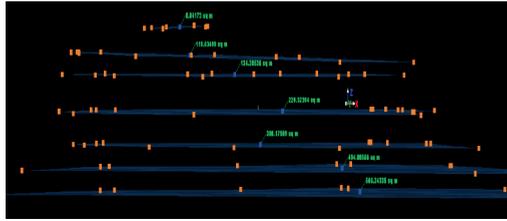


Figure 11. Values of the base areas of the elementary cylinders (Valley 7)

Using the values of the base areas of the 7 elementary cylinders in Figure 11 and the height between them, the volume of land

displaced for the 7th valley wire was calculated (Table 8).

Table 8. Volume calculation for valley wire 7

Plan number	Surface (m <sup>2</sup> )	Height (m)	Volume (m <sup>3</sup> )
1	8.04	1	8.04
2	119.63	1	119.63
3	134.38	1	134.38
4	229.32	1	229.32
5	300.17	1	300.17
6	494.8	1	494.8
7	566.34	1	566.34
<b>Total</b>			<b>1852.68</b>

Finally, in order to determine the value of the cubage of the entire form of negative relief, the values of the volumes of each valley were added:  $S_T = 1180.88 \text{ m}^3 + 3409.124 \text{ m}^3 + 340.38 \text{ m}^3 + 2055.4 \text{ m}^3 + 813.83 \text{ m}^3 + 1852.68 \text{ m}^3 + 10134.08 \text{ m}^3 = 20268.168 \text{ m}^3$ .

As can be seen from the value of the obtained cubage of  $20268.168 \text{ m}^3$ , the amount of soil displaced by the erosion phenomenon is very significant, along with it being removed and a significant amount of organic matter, and therefore carbon.

From the estimates of the Worldwatch Institute, it is estimated that in about 150 years the fertile soil reserves are depleted with an annual depletion rate of 23%. Careful conservation and terracing of sloping land can limit erosion. The quantitative evaluation of the erosion rate is made by experimental researches on the specially arranged plots, and the soil losses are measured in tons/ha/year or tons/km<sup>2</sup>/year. Japan is the country with the most sloping land, but has a low erosion rate. For example, on slopes with slopes below 10°, the erosion rate is 1 ton/ha/year. On the steeper slopes, over 10°, the erosion on bare lands (without vegetation) (Răduțoiu et al., 2018) can be of 20-40 t/ha/year, and on lands with vegetation reaches

less than 20 t/ha/year. The highest erosion rates in the world have been recorded in the Loess Plateau of China, where soil losses can reach 500 t/ha/year (Pop et al., 2019). In order to control the phenomenon of soil erosion, special control measures must be taken. These measures cannot be applied without a very clear and precise inventory of all areas prone to such a phenomenon. Inventory from the point of view of the surfaces and the volume of dislocated soil, can be done very easily and precisely by modern topo-geodetic methods, of the type presented in this paper.

## CONCLUSIONS

The topographic survey method presented is a modern, topical and high precision method, and can be used very easily to monitor and inventory land subject to erosion, both to determine the affected areas and to determine the volume of soil displaced. Research has shown that the method has a number of advantages compared to other methods used so far that it is recommended to be used in such works, such as: high accuracy, considerable reduction in time and costs of measuring and analyzing data, compared to classical

measurements; adequate and feasible equipment for measuring the volumes of embankments, ensuring a better vision of project financing, guaranteed quality of data and work performed, quantitative monitoring of any type of engineering work, flexibility to adapt to changes in the field, non-invasive method of taking over of data.

One aspect that clearly differs from the other methods is that the objective under study can be manipulated in the virtual environment, thus providing viewing angles, otherwise impossible. In this way, the original lens is protected, no longer requiring physical manipulation. It is also no longer necessary to travel to the location of the objective under study, its replica being accessible via the Internet. In addition, the digital replica with the information thus applied on its surface, represents a complex digitization of the objective, being able to remain testimony in time, in case of changing the state of conservation of the original objective. In conclusion, the terrestrial laser scanning technology presented can be used successfully as a complementary method to other lifting methods, but it can very successfully replace traditional geodetic methods of measurement, as it has the advantages listed above, which recommend it with great confidence.

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## FUEL CONSUMPTION AND CO<sub>2</sub> EMISSIONS IN SILAGE CORN PRODUCTION IN KOCAELI PROVINCE OF TURKEY

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### Abstract

*In this study, total fuel (diesel + motor oil) consumption, energy consumption and carbon dioxide (CO<sub>2</sub>) emission in silage maize production in Kocaeli region were evaluated. 195.145 L/ha fuel (Diesel + lubrication r oil) is consumed in usage of tools and machinery in silage maize production. For this process, a total of 7241.62 MJ/ha of diesel and motor oil consumption is made. In silage maize production, 535.92 kg CO<sub>2</sub> emission is produced regarding the consumption of 193.28 /ha diesel and 1,864 L/ha motor oil. In this region, 4,8447 L total fuel (Diesel + lubrication oil) and 179.782 MJ energy were consumed to produce 1 ton of silage maize. In result of total fuel consumption, 13.3049 kg CO<sub>2</sub> is released for 1 ton of silage maize production.*

**Key words:** Kocaeli, Silage maize, Fuel consumption, Energy consumption, CO<sub>2</sub> emission.

### INTRODUCTION

Carbon dioxide (CO<sub>2</sub>) comes first among the greenhouse gases and this effect is global. Some pollutants have local characteristics such as acid rains caused by SO<sub>2</sub> emission. Greenhouse gases are released through both natural processes and human activities. The most important natural greenhouse gas in the atmosphere is water vapor. However, human activities increase the atmospheric concentrations of these gases, causing large amounts of greenhouse gases to be released. This situation warms the climate by increasing the greenhouse effect.

According to the data of Turkish Statistical Institute (TUIK, 2019), total greenhouse gas emissions in Turkey was realized as 496.1 million tons of carbon dioxide equivalent in 2016. In this period, the largest share of CO<sub>2</sub> emissions in total emissions was energy-related emissions with 72.8%, followed by industrial processes and product use with 12.6%, agricultural activities with 11.4% and waste with 3.3%, respectively. Total greenhouse gas emissions in 2016 as CO<sub>2</sub> equivalent increased by 135.4% compared to 1990. While the carbon dioxide equivalent emission per capita was calculated as 3.8 tons/person in 1990, this value

was realized as 6.3 tons/person in 2016. In 2016, 33.1% of total CO<sub>2</sub> emissions were from electricity and heat production, 86.1% was from energy, 13.6% was from industrial processes and product use, 0.3% was from agricultural activities and waste. 55.5% of methane emissions originated from agricultural activities, 25.8% from waste, 18.6% from energy and 0.03% from industrial processes and product use. Agricultural activities constituted the biggest share in diazo monoxide (NO<sub>2</sub>) emissions with 77.6%. This was followed by energy with 12.1%, waste with 6.5%, and industrial processes and product use with 3.8%. In Turkish National Energy Efficiency Action Plan for the period between years 2017-2023 (UEVEP, 2017), 6 actions have been determined like to encourage the renewal and increase energy efficiency of tractors and combined harvesters to increase energy efficiency in the agriculture sector and introduction of energy-efficient irrigation methods, promotion of energy efficiency projects in the agricultural sector to encourage the use of renewable energy sources in agricultural production, to identify and promote the use of agricultural by-products and waste potential in order to obtain biomass, and to support energy efficiency in the aquaculture industry.

One of the important silage plants is maize. Maize silage is used extensively in the feeding of animals, calves, dry and lactating cows in modern farms. Maize silage should be backed with protein, mineral and sometimes energy to meet adequate nutrition requirements of the animals in question. The feeding strategy for maize silage given to beef cattle due to its high grain content differs from other roughages. Considering the balance of beef cattle rations, maize silage's important quality factors are; energy content, cellulose content, starch content and digestion degree. The supply of energy and protein constitutes a significant part of the beef cattle ration cost. Therefore, maize silage is the most logical alternative feed raw material for providing sufficient energy in beef cattle feeding programs (Tezel, 2018). When silage is mentioned all over the world, the first plant that comes to mind is maize. Maize cultivation and seed are easy to find. Production per unit area is high also it is suitable for mechanized agriculture. Feeding value is good. It is suitable for silage and has a high rate of soluble carbon hydrates and dry material. Therefore, it is easily fermented and maintains its quality for a long time (Tan, 2015).

In this study, it was aimed to determine the total energy consumption (diesel + motor oil) in silage maize production in Kocaeli and to determine the energy efficiency of the production. For this purpose, the processes and fuel consumption in the production of silage maize in the districts of Kocaeli region were examined in detail. The diesel consumption values of the tools and machinery used in the production of silage maize were determined by surveys conducted with experts in the Agriculture and Forestry District Directorates. During the process of silage maize production, efficiency criteria for total fuel (Diesel + lubrication oil) consumption for the tractor engine in the use of equipment and machinery are defined as follows, based on production and consumption and CO<sub>2</sub> emission values.

## MATERIALS AND METHODS

### Materials

Kocaeli is Turkey's tenth most populous province and is one of Turkey's largest industrial and commercial city. It is the third largest city

in Marmara Region after Istanbul and Bursa (Figure 3.1). As of 2019, it has a population of 1 906 391 people. It's name comes from Akcakoca, who conquered the region of Izmit in 1320. It is neighbor to Istanbul, Sakarya, Bilecik, Bursa and Yalova provinces. The area of the province is 3 397 km<sup>2</sup>. 539 people per km<sup>2</sup> in the province. The highest population ratio is in Darica with 8 310 people per km<sup>2</sup>. The annual population growth rate in the province was 2.85%.

### Geographical Features

Kocaeli province is located between 29°22'-30°21' East longitude, 40°31'-41°13' North latitude in the Catalca-Kocaeli side of the Marmara Region. It is surrounded by Sakarya on the east and southeast, Bursa on the south, Yalova province, Gulf of Izmit, Marmara Sea and Istanbul on the west, and the Black Sea on the North 30° East longitude, passing by the east of the city center of Izmit and time is considered as base for Turkish local time. The surface area of Kocaeli province is 3.505 km<sup>2</sup> and it is located at an important road junction connecting Asia and Europe.

### Crop Production

Population and land assets of Kocaeli districts are given in Table 1. Within the total area of 342 001 ha of the province, the cultivated area is 72 579 ha and it covers 21.2% of the province area. Within the total cultivated agricultural area, the field farming area is 56424.4 ha and the production is 450 870 tons, the fruit farming area is 12 548.1 ha and the production is 51 829 355 tons, the vegetable farming area is 3 166.5 ha and the production is 79 373 tons, the covered (green house) farming area is 314.8 ha and production is 17 512 314 tons.

Table 1. Population and Land Area of Kocaeli Districts

Dist.name	Population	Area (ha)
Basiskele	97 817	21 711.3
Derince	140 982	19 460.3
Dilovası	47 948	13 385.7
Gebze	371 000	42 514.5
Göleuk	162 584	22 629.4
Izmit	363 416	48 447.2
Kandıra	51 348	85 471.3
Karamürsel	56 604	25 460.1
Kartepe	118 066	26 913.8
Korfez	165 503	30 742.5
Çayırova	129 655	2 903.2
Darica	201 468	2 361.3
<b>Total</b>	<b>1 906 391</b>	<b>342 001.3</b>

In 2018, in Kocaeli, silage maize farming was made in 56 116 ha area. The total of 185 379 tons of maize has been cultivated and the output per hectare is 3 304 kg.

**Methods**

**Calculation of Total Fuel Consumption**

The diesel and engine oil values per unit production area (da) consumed by tractor during silage maize production processes were evaluated as total fuel consumption.

$$TFC = DC + MYT \dots\dots\dots(1)$$

Where:

*TFC* = Total fuel consumption (L/ha)

*DC* = Diesel consumption (L/ha)

*LOC* = Lubrication oil consumption (L/ha)

**Calculation of Lubricant Consumption**

Lubrication oil (lubricant) consumption per hour for tractor used in silage maize production operations was determined as follows, depending on the tractor's highest PTO power (Ozturk, 2010).

$$LOC = 0.00059 \times PTO_{max} + 0.02169 \dots\dots\dots(2)$$

Where:

*LOC* = Tractor lubrication oil consumption per hour (L/h)

*PTO<sub>max</sub>* = Tractor's highest PTO power (kW)

The maximum tail shaft power (*PTO<sub>max</sub>*) for the agricultural tractor used for silage maize production is taken into account as 88% of the tractor rated power (TRP, kW) and is determined as follows (Ozturk, 2010).

$$PTO_{max} = 0.88 \times TRP \dots\dots\dots(3)$$

Where;

*PTO<sub>max</sub>* = Tractor's highest *PTO* power (kW)

*TRP* = Tractor rated power (kW)

**Determination of Total Energy Consumption**

The total energy consumption (*TEC*, MJ/ha) pertaining to the consumption of diesel and engine oil per unit production area (da) was determined by the tractor used during silage maize production processes as follows.

$$TEC = DEC + LEC \dots\dots\dots(4)$$

Where

*TEC* = The total energy consumption (MJ/ha)

*DEC* = Diesel energy consumption (MJ/ha)

*LEC* = Lubrication oil energy consumption (MJ/ha)

**Calculation of Diesel Energy Consumption**

The diesel energy consumption (*DEC*, MJ / ha) related to diesel consumption consumed per

unit production area (ha) by the tractor used during silage maize production processes is determined as follows.

$$DEC = DC \times LHV_D \dots\dots\dots(5)$$

Where;

*DEC* = Diesel energy consumption (MJ/ha)

*DC* = Diesel consumption (L/ha)

*LHV<sub>D</sub>* = Lower Heating Value of Diesel (MJ/L)

The lower thermal value of the diesel (fuel) consumed by the tractor during agricultural production using agricultural tools and machinery was considered as 37.1 MJ/L (IPCC, 1996).

**Calculation of Lubrication Oil Energy Consumption**

The lubrication oil energy consumption (*LEC*, MJ/ha) per unit production area (ha) consumed by tractor used during silage maize production processes was determined as follows.

$$LEC = LOC \times LHV_L \dots\dots\dots(6)$$

Where;

*LEC* = Lubrication oil energy consumption (MJ/ha)

*LOC* = Lubrication oil consumption (L/ha)

*LHV<sub>L</sub>* = Lower Heating Value of lubrication oil (MJ/L)

The lower heat value of the lubrication oil consumed by the tractor during the production operations in the field area with agricultural tools and machinery was taken into account as 38.2 MJ/L (IPCC, 1996).

**Calculation of CO<sub>2</sub> Emissions**

The CO<sub>2</sub> emissions from all motor vehicles burning fossil fuels can be calculated taking into account the amount of fuel consumed and the distance travelled. In the method of calculating CO<sub>2</sub> emissions taking into account the amount of fuel consumed, the value of fuel consumption is multiplied by the CO<sub>2</sub> emission factor for each type of fuel. This emission factor is developed depending on the thermal value of the fuel and the carbon fraction oxidized in the fuel and the carbon content. This approach is defined as the fuel-based CO<sub>2</sub> emission calculation method as it uses average fuel consumption data. The fuel consumption-based approach can be applied taking into account vehicle effectiveness data and fuel economy factors that enable the calculation of fuel consumption. Distance-based emission factors are taken into account when calculating

emissions using the distance-based method. The fuel-based CO<sub>2</sub> emission calculation method is the preferred approach, since data on the fuel consumed is generally more reliable. However, since the uncertainty level in CO<sub>2</sub> estimates can be quite high, the distance based method should be used as a last remedy (IPCC, 1996).

### Fuel Heating Values and Selection of Emission Factors

Taking into consideration the lubrication oil consumption value of the tractor engine, CO<sub>2</sub> emissions related to oil consumption can also be calculated. The values given in Table 2 are used for the thermal values of diesel fuel and engine oil and CO<sub>2</sub> emission factors depending on the type of fuel.

Table 2. Thermal Values and CO<sub>2</sub> Emission Factors (IPCC, 1996)

Fuel	Lower heating value (MJ/L)	CO <sub>2</sub> Emission factor (kg CO <sub>2</sub> /MJ)
Diesel	37.1	0.07401
Lubricant oil	38.2	0.07328

### Calculation of total CO<sub>2</sub> Emissions

In calculating the CO<sub>2</sub> emissions released in result of silage maize production, the fuel-based CO<sub>2</sub> emission calculation method proposed in the Intergovernmental Panel on Climate Change was taken into account (IPCC, 1996). The proposed approach to calculate CO<sub>2</sub> emissions based on fuel consumption is summarized in equations (8) and (9).

The total CO<sub>2</sub> emission (*TCO<sub>2</sub>E*, kg CO<sub>2</sub>/ha) pertaining to the consumption of diesel and engine oil per unit production area (da) was determined by the tractor used during silage maize production processes as follows.

$$TCO_2E = CO_2ED + CO_2EL \dots \dots \dots (7)$$

Where;

*TCO<sub>2</sub>E* = Total CO<sub>2</sub> emission (kg CO<sub>2</sub>/ha)

*CO<sub>2</sub>ED* = CO<sub>2</sub> emission related to Diesel consumption (kgCO<sub>2</sub>/ha)

*CO<sub>2</sub>EL* = CO<sub>2</sub> emission related to lubricant oil consumption (kgCO<sub>2</sub>/ha)

### Calculation of CO<sub>2</sub> Emission Related to Diesel Consumption

The CO<sub>2</sub> emission (*CO<sub>2</sub>ED*, kg CO<sub>2</sub>/ha) for diesel consumption per unit production area (ha) was determined by the tractor used during silage maize production processes as follows:

$$CO_2ED = DC \times LHV_D \times EF_D \dots \dots \dots (8)$$

Where:

*CO<sub>2</sub>ED* = The CO<sub>2</sub> emission related to Diesel consumption (kg CO<sub>2</sub>/ha)

*DC* = Diesel consumption (L/ha)

*LHV<sub>D</sub>* = Lower Heating Value of Diesel fuel

*EF<sub>D</sub>* = The CO<sub>2</sub> emission factor for Diesel fuel (0.07401 kg CO<sub>2</sub>/MJ)

### CO<sub>2</sub> Emission Calculation Related to Lubrication Oil Consumption

The CO<sub>2</sub> emission (*CO<sub>2</sub>EL*, kg CO<sub>2</sub>/ha) related to engine oil consumption per unit production area (ha) was determined by the tractor used during silage maize production processes as follows.

$$CO_2EL = LOC \times LHV_L \times EF_L \dots \dots \dots (9)$$

where:

*CO<sub>2</sub>EL* = The CO<sub>2</sub> emission related to lubrication oil consumption (kg CO<sub>2</sub>/ha)

*LOC* = Lubrication oil consumption (L/ha)

*LHV<sub>L</sub>* = Lower Heating Value of lubrication oil (38.2 MJ/L)

*EF<sub>L</sub>* = The CO<sub>2</sub> emission factor for lubrication oil (0.07401 kg CO<sub>2</sub>/MJ)

## RESULTS AND DISCUSSIONS

### Total Fuel Consumption on Silage Maize Production

During silage maize production processes, the sum of the diesel and engine oil consumption values consumed by the tractor engine in the use of tools and machinery was taken into account as the total fuel consumption. During silage maize production processes, the change in the total fuel consumption values related to the use of tools and machinery are given in Table 3.

The total fuel consumption values given in Table 3 indicate the average values of the total fuel consumption values determined from the districts of Kocaeli. It is seen that the total fuel consumption values are in parallel with the change in the usage time of the tools and machines used in the silage maize production processes and the loading rates of the tractor engine. The highest total fuel consumption occurs in silage maize harvesting operations with value of 33.422 L per unit area (ha). The second place in total fuel consumption is plough tillage applications with a value of 26.695 L/ha. The total fuel consumption in silage maize production, 23.61 L/ha in sowing

process with planting machine, 21.77 L/ha in fertilizer intermediate hoeing process, 20.69 L/ha in sprout with disc harrow, 21.98 L/ha in fertilizer distribution process is 19.816 L/ha in cultivation with cultivator, 18.559 L/ha in spraying with sprayer, and 10.283 L/ha in scaling. A total of 195.145 L/ha fuel (diesel + lubrication oil) is consumed in the use of tools and machinery in silage maize production.

Table 3. The Total Fuel Consumption Values in Silage Maize Production

Equipments/ Machines	Fuel Consumption (L/ha)		
	Diesel consumption (L/ha)	Lubricant consumption (L/ha)	Total (L/ha)
Plough	26.44	0.255	26.695
Cultivator	19.63	0.186	19.816
Disc harrow	20.69	0.198	20.888
Chisel plough	10.19	0.093	10.283
Fertilizer sprayer	19.70	0.192	19.892
Planter	23.38	0.230	23.610
Fertilizer distributor with hoeing	21.77	0.210	21.980
Sprayer	18.38	0.179	18.559
Harvester	33.10	0.322	33.422
<b>Total</b>	<b>193.28</b>	<b>1.864</b>	<b>195.145</b>

### Total Energy Consumption Related to Total Fuel Consumption

In the process of silage maize production, the sum of the energy values related to the Diesel and lubrication oil consumption consumed by the tractor engine in the use of tools machinery was taken into account as the total energy consumption related to the total fuel consumption. In the process of silage maize production processes, the change of the total energy consumption values related to the total fuel consumption related to the use of tools and machinery are given in Table 4.

Table 4. Energy Consumption Related to Fuel Consumption in Silage Maize Production

Equipments/ Machines	Energy consumption(MJ/ha)		
	Fuel consumption (MJ/ha)	Lubricant consumption (MJ/ha)	Total (MJ/ha)
Plough	981.05	9.75	990.80
Cultivator	728.26	7.11	735.37
Disc harrow	767.75	7.57	775.32
Chisel plough	377.87	3.54	381.41
Fertilizer spreader	730.96	7.35	738.31
Planter	867.34	8.78	876.12
Fertilizer distributor with hoeing	807.24	8.03	815.27
Sprayer	681.84	6.83	688.67
Harvester	1228.06	12.29	1240.35
<b>Total</b>	<b>7170.37</b>	<b>71.25</b>	<b>7241.62</b>

Energy consumption values related to total fuel consumption given in Table. 4 indicate the average values of energy consumption values for total fuel consumption determined from the districts of Kocaeli. It is seen that the total energy consumption values related to the total fuel consumption are in parallel with the change in the usage time of the tools and machines used in the silage maize production processes and the loading rates of the tractor engine. Total energy consumption related to the highest total fuel consumption is realized in silage maize harvesting with 1 240,35 MJ per unit area (ha). The second place in energy consumption regarding total fuel consumption is plough tillage applications with a value of 990.8 MJ/ha. Total energy consumption for total fuel consumption in silage maize production is 876.12 MJ/ha, respectively in cultivation process 815.27 MJ/ha, in fertilizer intermediate hoeing process, 775.32 MJ/ha, in cultivation of the disc harrow, 738 MJ/ha, in the fertilizer distribution process, 31 MJ/ha, 735.37 MJ/ha in cultivation with cultivator, 688.67 MJ/ha in spraying with sprayer, and finally 381.41 MJ/ha in plough cultivation. For the use of tools and machinery in silage maize production, a total of 7 241.62 MJ/ha of Diesel and lubrication oil consumption is made.

### The Total CO<sub>2</sub> Emission Related to Total Fuel Consumption

In silage maize production processes, the total CO<sub>2</sub> emission values regarding the Diesel and lubrication consumption made by the tractor engine in the use of tools and machinery were taken into account as the total CO<sub>2</sub> emission related to the total fuel consumption. In silage maize production processes, the change in the total CO<sub>2</sub> emission values related to the total fuel consumption related to the use of tools and machinery are given in Table.5.

The CO<sub>2</sub> emission values for total fuel consumption given in Table 5 indicate the average values of CO<sub>2</sub> emission values for total fuel consumption determined from the districts of Kocaeli province.

It is observed that the total CO<sub>2</sub> emission values related to the total fuel consumption are in parallel with the change in the usage time of the tools and machines used in the process of silage maize production and the loading rates of the tractor engine.

Table 5. The CO<sub>2</sub> Emission Related to Fuel Consumption in Silage Maize Production

Equipments/ Machines	CO <sub>2</sub> Emission (kgCO <sub>2</sub> /ha)		
	Fuel consumption (kg CO <sub>2</sub> /ha)	Lubrication (kg CO <sub>2</sub> /ha)	Total (kg CO <sub>2</sub> /ha)
Plough	72.61	0.715	73.33
Cultivator	53.90	0.521	54.42
Disc harrow	56.82	0.555	57.38
Chisel plough	27.97	0.259	28.23
Fertilizer spreader	54.10	0.539	54.64
Planter	64.19	0.645	64.84
Fertilizer distributor with hoeing	59.74	0.589	60.33
Sprayers	50.46	0.501	50.96
Harvesting	90.89	0.900	91.79
<b>Total</b>	<b>530.68</b>	<b>5.224</b>	<b>535.92</b>

The highest total CO<sub>2</sub> emission related to total fuel consumption is realized in silage maize harvesting processes with 91.79 kg CO<sub>2</sub> per unit area (ha). The second place in CO<sub>2</sub> emission related to total fuel consumption is plough tillage applications with a value of 73.33 kg CO<sub>2</sub>/ha. Total CO<sub>2</sub> emission related to total fuel consumption in silage maize production, 64.84 kg CO<sub>2</sub>/ha in sowing process, 60.33 kg CO<sub>2</sub>/ha in fertilizer intermediate hoeing process, 57.38 kg CO<sub>2</sub>/ha in soil cultivation with disc harrow, 54 in fertilizer distribution process, 64 kg CO<sub>2</sub>/ha, 54.22 kg CO<sub>2</sub>/ha in tillage, 50.96 kg CO<sub>2</sub>/ha in spraying with sprayer and 28.23 kg CO<sub>2</sub>/ha in tillage. In the use of tools and machinery in the production of maize silage, 535.92 kg of CO<sub>2</sub> emission is realized regarding the consumption of 193.28 L/ha Diesel and 1.864 L/ha lubrication oil.

## CONCLUSIONS

Total consumption of fuel (diesel+lubrication oil) in silage maize production is 33.422 L/ha in harvesting process with silage machine, 26.695 L/ha in tillage with plough, 23.61 L/ha in planting machine, 21.77 L/ha in fertilizer cultivation, 20.69 L/ha in soil tillage with disc harrow, 21.98 L/ha in fertilizer distribution,

19.816 L/ha in tillage with cultivator, 18.559 L/ha in spraying with sprayer, and finally in tillage with chisel plough it is realized as 10.283 L/ha.

The total energy consumption for total fuel consumption in silage maize production is 1240.35 MJ/ha in harvesting process with silage machine, 990.8 MJ/ha in tillage with plough, 876.12 MJ/ha in sowing with seed drill, intermediate hoeing with fertilizer 815.27 MJ/ha, 775.32 MJ/ha in tillage with disc harrow, 738.31 MJ/ha in fertilizer distribution, 735.37 MJ/ha in tillage, 688.67 MJ/ha in spraying with sprayer and in land cultivation, it is 381.41 MJ/ha.

The total CO<sub>2</sub> emission related to total fuel consumption in silage maize production, 91.79 kg CO<sub>2</sub>/ha in harvesting process with silage machine, 73.33 kg CO<sub>2</sub>/ha in soil cultivation with plough, 64.84 kg CO<sub>2</sub>/ha in cultivation with seed drill, 64.84 kg CO<sub>2</sub>/ha in fertilizer cultivation 60.33 kg CO<sub>2</sub>/ha, 57.38 kg CO<sub>2</sub>/ha in soil cultivation with disc harrow, 54.64 kg CO<sub>2</sub>/ha in fertilizer distribution, 54.22 kg CO<sub>2</sub>/ha in cultivation with cultivator, 50.96 kg CO<sub>2</sub>/ha in spraying and 28.23 kg CO<sub>2</sub>/ha in soil cultivation with chisel plough.

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## ROMANIAN AGRICULTURAL MARKET: THE RELATIONSHIP BETWEEN ECONOMIC GROWTH, ORGANIC PRODUCTION AND THE LABOUR MARKET

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### Abstract

*The importance of the agricultural sector, of the organic production and of the agricultural labour market leads the authors to study their dynamics and characteristics with regard to Romania. Food safety is linked to the agricultural activity's robustness and its evolution, given that the primary sector is the one that defines economic growth. The paper studies the link between the economic growth and the organic agricultural production in Romania, including labour market's characteristics. The time period is 2010-2019, and the methodology is that of a cross-sectional study. The multiple regression model is used to analyse the relationship between Romania's economic growth and seven factors which characterize the agricultural sector. The results show that, between Romania's economic growth and the variables under study, there is a link that is maintained over time, and which is positive in some cases and negative in other cases. Agricultural technology decreases the role and contribution of salaried and non-salaried labour in agriculture to Romanian economic growth. Processing of agricultural products has the same effect on growth. Instead, the authors outline the important role of organic agriculture, of agricultural services and of livestock production in the Romanian economy.*

**Key words:** *agricultural labour market, agricultural sector, economic growth, organic production.*

### INTRODUCTION

The paper's main goal is to study the link between economic growth and agricultural production, mainly the organic one, in Romania, also by analysing the contribution of salaried and non-salaried labour in the primary sector.

In UE, 50% of the territory is represented by rural areas with a population of 113 million of inhabitants and providing 20% of the work places, and 15% of the gross value added (GVA) in European economy (Paul, 2020). Agriculture remains a prevailing activity in European rural areas, in spite of the economic, social, political and environmental changes happening in the agricultural sector (Todorova & Ikova, 2014). The agricultural sector has significant differences between the European regions (Guth & Smędzik-Ambroży, 2019), and agricultural sustainability is moderate. The Central European states have the largest contribution to the economy, while the Mediterranean states have the largest contribution to the environment, and the

Eastern European states mostly contribute to employment generation (Dos Santos & Ahmad, 2020). Agriculture has a complex multiplier effect in all sectors of the economy, being a supplier and consumer for a wide range of products (Andrei et al., 2017). European agriculture has little contribution to GDP, about 1.6%; it provides 4.5% of jobs and 1.2% of exports and 1.4% of imports (Dos Santos & Nawaz, 2020). However, the pressures on the environment are important. In UE, according to Renner et al. (2020), half of the greenhouse gas emissions, other than CO<sub>2</sub>, are generated by agricultural activities. UE imports a significant number of agricultural products, with imports exceeding exports in terms of aggregate quantity. The labour force involved in agriculture is scarce. Three quarters of the European population live in urban areas, and the role of European farmers is to feed the urban population. Renner et al. (2020) argue that the reduction of the agricultural workforce is a recent trend that is not easy to reverse, and that technical progress will not be enough to deal with future challenges. Another trend,

according to Fitton et al. (2019), is the reduction of agricultural land simultaneously with a change in the attitude towards food, in the sense of reducing food waste. This is the biggest buffer against food insecurity. Agricultural productivity has increased progressively, but with an impact on the ecosystem (Fitton et al., 2019), causing biodiversity loss (Rega et al., 2020). Climate change influenced global agriculture. Prăvălie et al. (2020) show that, between 1980 and 2008, maize production decreased globally by an average 3.1% because of temperature change, and by 0.7% because of changes in rainfall. Agriculture follows patterns of seasonality correlated with product's life cycle, climate and physical characteristics of the land (Rembold et al., 2019). On the long term, reducing cereal crop production will affect food security and socio-economic stability. All these trends were in favour of the development of organic farming. The demand for organic products has increased substantially in Europe; organic production has become a lifestyle (Bejinaru et al., 2020) and it is the solution to solve environmental problems such as global warming, biodiversity reduction and desertification (Stoi et al., 2020).

Romania still has difficulties in reaching the socio-economic potential of the agricultural sector and rural areas, inhabited by 46.3% of the population in 2018 (Răzvanță Puie, 2020). The Romanian agricultural sector is of utmost importance for the economy and society. Romania is considered the most rural European country. Nevertheless, it lacks diversification, it relies almost exclusively on relatively small farms, it lacks technologically advanced equipment, storage for primary production, packaging and transport possibilities (Paul, 2020).

The agricultural structures in Romania are characterized by a dual model: most farms are relatively small, and the used agricultural area is half of the total land (Wolz et al., 2020). The Romanian agricultural system has undergone important transformations in terms of land use, productivity and operation, and it is still a sector of utmost importance for the Romanian economy, which can be described as agrarian economy, with an impact on rural communities (Andrei et al., 2017). Cereal crops, mostly

wheat and maize, are important in Romania's agriculture, according to Buliga-Ștefănescu (2019). The author argues that, in spite of the fact that Romania has a significant agricultural potential, it cannot be used adequately because of the lack of mechanization, the fragmentation, the lack of capital and of irrigation systems, and farmers' lack of formal training and their old age.

As a EU member state, Romania strengthens its position in agriculture, especially with the increase of the percentage of land intended for organic production, which can raise the net revenues and lower the negative impact on environment and the risks associated to weak crops (Creceană & Creceană, 2019).

The added value of organic agriculture is important especially for the local economy; however, there is low demand for organic products (Ak & Teker, 2020). Still, the literature also draws attention to the opposite side, namely that the agricultural products industry has exploded, the demand is higher than ever (Nain et al., 2020) and growing (Nikolova, 2019), and the expansion on the European market was 200% during 2000–2017 (Blaće et al., 2020). Creceană & Creceană (2019) show that the role of organic agriculture in the future depends on its productivity and capacity to become economically competitive with conventional agriculture. This depends on the organic agriculture productivity, the demand and if the sale prices reflect the cost of outsourcing linked to production reorientation, including those linked to environment and health.

The decision to consume organic agricultural products follows the awareness regarding the environmental issues, and women are most likely to purchase them (Do Prado, 2020). A study of the factors influencing the dynamics of organic farming development in Croatia shows that this type of farming is adopted based on lifestyle, but also on the environment, ideology and philosophy of producers and consumers, regardless of age or educational level (Blaće et al., 2020).

Romania has great potential for organic farming even if the number of certificates is low for the time being. This market segment needs to be exploited due to the increasing European and global demand for organic

products with high added value (Creacă & Creacă, 2019). The future importance of this niche market means that rural areas play a key role in the development of the bio-economy, which covers all innovative production activities that use the conversion of biological resources (Butu et al., 2020), as agriculture is oriented towards more environmentally responsible methods (Popescu & Safta, 2020). Given the agriculture's importance for the European and Romanian economy, for food, social, political and institutional safety, as well as the increasing role and weight of organic production, the authors propose an analysis of the Romanian agricultural market in relation to economic growth, on one hand, and to organic production and labour market, on the other hand. Four hypotheses were formulated and the multiple linear regression for time series was used.

H1: organic agriculture has a positive impact on Romania's economic growth;

H2: agricultural services and livestock production have a positive impact on Romania's economic growth;

H3: salaried and non-salaried labour force has a positive impact on Romania's economic growth;

H4: cereal production and the processing of agricultural products have a positive impact on Romania's economic growth.

The paper's sections show the analysed indicators, used methods, results and conclusions, outlining the research's usefulness and limitations.

## **MATERIALS AND METHODS**

### ***Data***

Data is retrieved from Eurostat and TEMPO, and the time period is 2010-2019. Data for 2019 are estimates. Labour market is described using indicators about labour force in agriculture, non-salaried labour force (NSLF) and salaried labour force (SLF). The measuring unit of these indicators is 1000 AWU (Annual Work Units). According to Eurostat ([https://ec.europa.eu/eurostat/statistics-explained/index.php/Glossary:Annual\\_work\\_unit\\_%28AWU%29](https://ec.europa.eu/eurostat/statistics-explained/index.php/Glossary:Annual_work_unit_%28AWU%29)), AWU is the equivalent of full-time employment or the total hours worked divided by the mean hours worked in the full-

time workplaces in the country. An annual work unit corresponds to the work carried out by a person employed full-time in an agricultural holding. Employment contracts regulate the minimum number of full-time working hours. If the employment contract does not specify the annual number of hours, then the minimum amount shall be deemed to be 1,800 hours, or the equivalent of 225 working days on an 8-hour basis. Given that the volume of agricultural work is calculated on the basis of full-time equivalents, no person can equalize more than 1 AWU. This restriction stays valid even if a person works in agriculture more hours than the legal number of hours considered as full-time.

Economic progress or growth is assessed with the help of gross domestic product (GDP) at current prices, expressed per capita. It characterizes the economic situation of an area and it represents the total value of goods and services produced over a given period of time, less the intermediate consumption consisting of all goods and services used to produce the former ones. We opted to express GDP in current prices because this eliminates price differences between countries and per capita values allow significantly different economies to be compared in terms of their size.

GDP per capita is expressed in national currency at current prices. In order to describe Romania's agricultural production, reference is made to the cereal production, including seeds (cer), livestock production (anim), agricultural services (serv) and processing of agricultural products (pap). The value of these components of agricultural production is rendered by the producer's price, which is divided by the number of inhabitants, which gives the value per capita.

The total Utilized Agricultural Area (UAA) measures, according to Eurostat (the database from which the indicator was retrieved), the percentage from the total agricultural area utilized for organic agriculture. Organic agriculture refers to organic farms in Romania and the farms in transition to organic agriculture. Farms are considered organic if they are aligned to Council Regulation (EC) No. 834/2007, which provides the framework for organic production, labelling and

processing, and whether they meet European import criteria.

**Methodology**

The method used to analyse the impact of agricultural product and labour market on Romanian economy is time series multiple linear regression. The time component was introduced into the multiple regression equation. The initial equation was of the following form:

$$Y_{it} = a + b \cdot X_{it} + \epsilon_t \quad (1)$$

Equation (1), customized for our analysis, was converted to Equation (2), where GDP is the dependant variable or the output, and uaa, cer, anim, serv, pap, slf and nslf are the seven independent or explanatory variables of the model.

$$GDP = \beta_0 + \beta_1 uaa + \beta_2 cer + \beta_3 anim + \beta_4 serv + \beta_5 pap + \beta_6 slf + \beta_7 nslf \quad (2)$$

Existing data were logarithmized in order to obtain more objective results.

**RESULTS AND DISCUSSIONS**

After data processing, the panel regression equation is as follows:

$$GDP = 32.97 + 0.45 \cdot uaa - 0.31 \cdot cer + 0.39 \cdot anim + 0.35 \cdot serv - 0.94 \cdot pap - 1.39 \cdot slf - 1.66 \cdot nslf \quad (3)$$

In the analysed model, the value of the coefficient of determination shows that the model matches the data very well. The economic growth variation is explained at 99.67% by the variation in the independent variables. There is a positive link between the economic growth and the agricultural area used for organic production (uaa), livestock production (anim) and agricultural services (serv), and a negative link between the economic growth and cereal and seed production (cer), processing of agricultural products (paps), and salaried labour force (slf) and non-salaried labour force (nslf). The negative link is explained to a great extent by

the introduction of agricultural technology and the shrinkage of processing industry (Buliga-Ştefănescu, 2019; Iancu et al., 2020), in spite of the fact that the small size of the majority of farms hinder the amortisation of technology investments (Salih, 2020). They remain the privilege of the large Romanian farms. However, for the year 2030, the OECD estimates that half of the agricultural production will be obtained using biotechnology (OECD, 2009; Butu et al., 2020), elements that combine technology with organic production and which help us anticipate the trend of agriculture mechanization and massive replacement of the labour force.

The values of the non-standardized coefficients show that an increase by one unit of the area intended for organic agriculture determines a GDP increase by 0.45 units, while the other factors remain unchanged, which supports the first hypothesis (H1). The increase of the livestock production by one unit results in the increase of GDP by 0.39 units, and the increase of the agricultural services by one unit leads to the increase of GDP by 0.35 units, which validates the second hypothesis (H2). In regard to the cereal production, the processing of agricultural products, the salaried and non-salaried labour force, their increase by one unit causes the decrease of GDP by 0.31 units, by 1.39 units, and by 1.66 units, respectively, which invalidates the third and the fourth hypotheses (H3, H4). The coefficients of the estimated model belong to confidence intervals, and p-value and t-value are according to Table 1.

Also, we determined the marginal value that production and labour market specific to the Romanian agricultural sector have upon economic growth: 10.493.

The values of variables describing the model show a good match. Graph 1 shows the relationships between variables. They are not very correlated with each other, as the model premises request.

The correlation coefficients (Table 2) show that there is a low-intensity negative relationship between the growth and the number of workers (salaried and non-salaried) and the livestock production, except for non-salaried workers, where the link is medium to high.

Table 1. Descriptive values of the model

Non-standardized coefficients		95% Confidence intervals	
Name	Value		
uaa	0.4476649	-0.3007815	1.196111
cer	-0.3134144	-0.6991974	0.0723686
anim	0.3896691	-1.041067	1.820405
serv	0.3463993	-0.0590851	0.7518838
pap	-0.9380073	-1.779361	-0.0966538
slf	-1.392845	-2.471694	-0.3139959
nslf	-1.657403	-3.420513	0.105708
cons	32.97539	14.78772	51.16306
<b>R-square</b>	0.9967	<b>Prob&gt;F</b>	0.0114>0.1
<b>Adj. R-square</b>	0.9853		
t-value		Theoretical t-value	
uaa	2.57	4.587	
cer	-3.5		
anim	1.17		
serv	3.68		
pap	-4.80		
slf	-5.55		
nslf	-4.04		
cons	7.8		

Source: authors' calculations in STATA

Table 2. Correlation coefficients

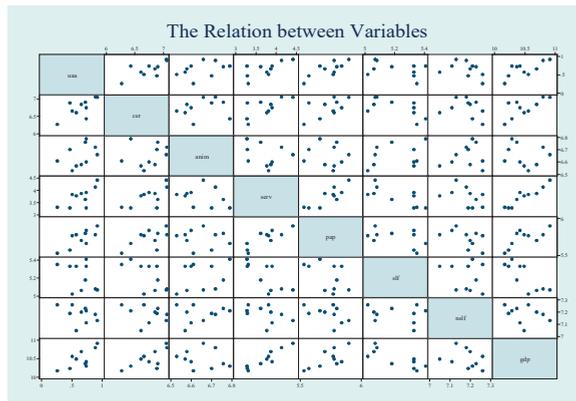
	uaa	cer	anim	serv	pap	slf	nslf
uaa	1.0000						
cer	0.7010	1.0000					
anim	0.4078	0.1834	1.0000				
serv	0.7041	0.6935	-0.2611	1.0000			
pap	0.7715	0.5932	-0.0991	0.8418	1.0000		
slf	-0.3838	-0.6307	0.2751	-0.7172	-0.4956	1.0000	
nslf	-0.3872	-0.2632	0.1761	-0.4307	-0.5106	-0.2043	1.0000
gdp	0.7169	0.7039	-0.1775	0.9435	0.7193	-0.7997	-0.3125

Source: authors' calculations

There are negative relationships between agricultural services and livestock production, between the processing of agricultural products and the processing of animal products, between salaried labour and the total area utilized for organic agriculture, but also between the last one and the cereal production, the agricultural services and the processing of agricultural services. There is also a negative relationship between non-salaried labour and the rest of explanatory variables, except for livestock production, where there is a low-intensity positive relationship. As for the livestock production and the area utilized for organic agriculture production, the relationship between variables is positive, with a low-to-medium intensity. The relationship between agricultural services and the area utilized for organic agriculture, between agricultural services and cereal production, but also between the processing of agricultural products and the organic agricul-

ture area and between the cereal production and the agricultural services is also positive, but its intensity is medium to high. The relationship between growth and explanatory variables is positive, of a medium-to-high intensity, except for livestock production, where it is negative and of very low intensity (-17.75%), non-salaried labour, where the intensity is medium to high (-31.25%), and salaried labour, where it is high (-79.97%).

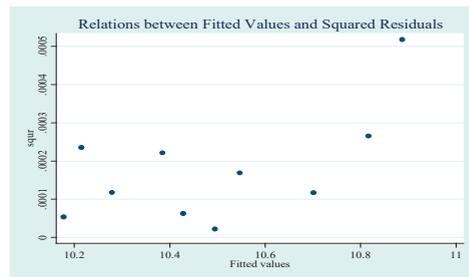
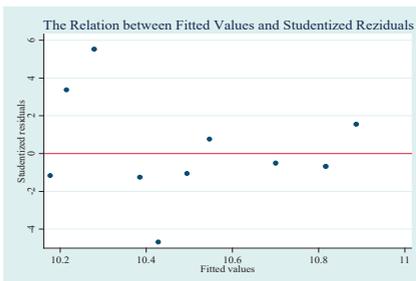
The relationship is positive and of medium-to-high intensity between growth and the area utilized for organic production (71.69%), between growth and cereal production (70.39%), and between growth and the processing of agricultural products (71.93%). The relationship is positive and of high intensity between economic growth and agricultural services (94.35%) (Table 1 in Annex). These results validate the hypotheses H1 and H2 and invalidates the hypotheses H3 and H4.



Graph 1. The relationship between variables

The VIF factor was used in order to identify multicollinearity issues. According to the resulted values, such issues are not identified in regard to the agricultural services (VIF=12.68), the area utilized for organic agriculture (12.30), and the salaried labour (14.00), but they are possible in all the other situations. With a mean value VIF = 9.42, the existence of some aspects linked to multicollinearity cannot be excluded

for the rest of the explanatory variables. Graph 2 is a verification of the assumptions on residual values in order to see whether the homoscedasticity condition is fulfilled, and the extreme cases were detected. Residual values are within the optimum interval, except for the ones which are specific to years 2011, 2012, and 2014.



Graph 2. The relationships between fitted values, studentized residuals, and squared residuals

Skewness (0.378) and Kurtosis (1.59) values show a normal distribution of the residuals. In regard to the Kurtosis value, there are two considerations. The repartition can be considered as slightly leptokurtic or it can be considered normal starting from the assumption that the kurtosis excess appears when the indicator's value exceeds 3, which is not the case. The Skewness/Kurtosis test validates the normality hypothesis, because the Prob>Chi value of 0.2263 exceeds  $\alpha$  (0.05), and the Shapiro-Wilk test strengthens this conclusion with the Prob>z value of 0.17639. The Breusch-Pagan test values ( $\chi^2(1) = 1.02$  and Prob> $\chi^2 = 0.3127$ ) and the bivariate and

multivariate normality test values (Prob> $\chi^2 = 0.1017$ ) validate the normality hypotheses. Therefore, this model matches the analysed data, validates the H1 and H2 hypotheses, and invalidates the H3 and H4 hypotheses. In spite of the high potential, the Romanian agriculture's competitiveness level is low compared to other European states, some of them with a lower potential (Andrei et al., 2020), given that 46.3% of the population is rural, the agriculture provides 29% of the total work places, but its GDP contribution is merely 5% (Faludi & Neamtu, 2020). The share of the agricultural economy in the rural economy is 60%, 4 times higher than in the EU, where it

merely reaches 14% (Boghean & State, 2020). The agricultural structure is unfavourable, less similar to that of European countries and closer to that of Latin America (Burja & Burja, 2016). Romania is the European country with the largest number of nonuniform farms, where the small ones prevail, but there are also large ones, outlining a weak structure. In addition, there are various shortcomings: low salaries compared to the salaries in Europe's developed countries, lack of training of farmers, lack of communication skills, which affects the managerial environment in the agricultural sector (Salih, 2020). Nevertheless, there have been signs of positive evolution especially after the year 2000. Between 2000 and 2018, labour productivity in agriculture almost doubled (Melnic & Puiu, 2019). Agricultural yields depend on climate variations, especially rainfall, which were insufficient in recent years, and the irrigation systems are not enough. Sugar beet crop was low in 2004, but it was one of the most productive years in regard to the cereal production which increased constantly, except for 2009, 2012 and 2015, thus being in opposition with the results of this research which invalidated hypothesis H4. This is due to the fact that the hypothesis combines the role of cereal production and of the processing of agricultural products in economic growth; the processing of agricultural products is severely short and has a negative impact on economic growth. An upward trend was reported in vegetable production, as well, as a result of subsidies for mechanization. Buliga-Ştefănescu (2019) shows that, after joining the EU, all types of crops increased their production due to the subsidies. Subsidies helped farmers to purchase the necessary technologies and products, fertilizers and herbicides, which were affordable especially for large producers. These are performances for Romanian agriculture, nevertheless, the yields are still lower than the European average.

In 2018, Romania had 30% of Europe's maize production, but, according to Prăvălie et al. (2020), climate change will affect Romania's position in the European hierarchy, because there is currently an acute shortage of irrigation infrastructure. Romania's cereal production is growing, but there are major concerns worldwide. Cereal production is the most

important pillar of global food safety and it should increase by 70% by 2050, in order to cope with the population growth (Prăvălie et al., 2020), which stimulates Romania to maintain its position regarding this crop on the European market. While the cereal and vegetable production increased, the animal production decreased by 1.76%, and the animal products decreased by 1.11% between 2015 and 2016 (Creacă & Creacă, 2019).

We have shown that Romania has potential for the development of organic agriculture because, in addition to the appropriate area, small amounts of fertilizers and chemicals are used, and an adequate green marketing strategy stimulates the production and consumption of organic products and helps develop the market and the agricultural sector (Aceleanu, 2016). Romania does not rely exclusively on conventional agriculture; it is oriented towards organic agriculture, which has become an important sector both in terms of demand and supply (Girip et al., 2020). Even though it started as a niche activity, with luxury products, intended for high-income social categories, organic agriculture tends to become the norm in the EU (Creacă & Creacă, 2019). In 2018, Romanian organic agriculture utilized an area of approximately 327 thousand hectares and involved 9008 farmers, where cereal and industrial crops prevailed (Şonea et al., 2020). Organically cultivated areas increased significantly between 2000 and 2017. Organic agriculture, through its mechanisms, favours the agricultural labour market.

In Romania, agricultural labour force increased, and after 1990 the individual, small farms, became dominant (Swinnen et al., 2005). Therefore, labour market is characterized by imbalances (Paşnicu & Ciucă, 2020), especially through low productivity (Burja & Burja, 2016; Buliga-Ştefănescu, 2019; Boghean & State, 2020). In spite of the many efforts, the outcome leaves much to be desired (Popescu, 2015). Agricultural labour is different from other sector's labour due to the following: it is non-uniform and unequal, the share of employed population is high, reaching 27% of the total employed population (Salih, 2020), and the education level is low. After 1990, agriculture incorporated the labour force made redundant from other sectors, acting like a buffer in the

labour market. According to certain sources, 12.7% of the agricultural workers are employed with a legal contract (Iancu et al., 2020), while other sources report a share of 16% (Salih, 2020). These values justify the low costs which stimulated the agricultural production based on the extensive use of labour, which resulted in the productivity decrease (Iancu et al., 2020). The Romanian rural environment is little diversified in terms of economy, so that local people are forced to practice subsistence agriculture (Faludi & Neamtu, 2020). In 2019 only 11.3% of the rural population had access to sewerage, 40% of households had constant access to drinking water, over 50% of the water in rural areas is not suitable for drinking, only 15% of the road infrastructure is modernized (Paul, 2020). This explains skills shortages, high unemployment rates and the dependence of the rural population on agricultural activities (Pleşca et al., 2020). There is a significant relationship between farmers' education, farm size, structure, and efficiency (Burja & Burja, 2016). Specialization helps increase efficiency, but small farms use low-skilled labour force. The low level of training of the labour force is an issue for Romanian agriculture. A share of 96.4% of the agriculture workers underwent on-the-spot training, compared to the European average of 70.95% (Salih, 2020). Salih (2020) also notices young people's lack of interest to gain knowledge and skills through training programs or other types of formal education. However, in spite of the very low performance, there has been progress due to the macro-economic stabilization programs, especially the ones involving privatisation, restructuring and liquidation of non-profitable activities, which allowed the recovery after 2000 (Rodriguez-Planas & Benus, 2010). Work productivity increased by 1.84 times between 2009 and 2018, and Romania scored one of the best results in Europe following the implementation of active measures targeted mostly at improving women participation to labour market, reducing employment in subsistence agriculture, involving more young people in labour market, supporting labour mobility locally, regionally and transnationally (Melnic & Puiu, 2019).

Our research shows the increased impact of agricultural sector on Romania's economic

growth. In spite of the existing shortcomings, taking appropriate measures, not only regarding the structural macroeconomic policy, but also the individual farm, on reorganization into various forms of associations, will help reduce costs through investment and technology. The irrigation system also needs to be developed, along with agriculture fertilization and mechanization, to reduce the dependence on climatic conditions. Industry development will help increase the export of processed products, leading to an increased contribution of agriculture to GDP, as the primary products export has low contribution due to low prices on the global market.

## CONCLUSIONS

The conclusion of our work is that Romania's economic growth between 2010 and 2019 received positive influence from the organic agriculture, analysed under the form of the area utilized by the organic farms. The same effect came from the livestock production and agricultural services. Between 2010 and 2019, the economic growth was negatively influenced by the processing of agricultural services, but also by the work of salaried and non-salaried people in the agricultural sector. Together, these two variables have a negative effect on economic growth; we have the certainty that cereal production's positive effect is neutralized by the negative effect of the processing of agricultural products, where Romania has a high deficit. Therefore, the orientation towards organic agriculture has positive effects on Romania's economic performance. The results encourage the development of this niche market, the improvement of agricultural services and of the livestock production. In regard to the agricultural labour market and the processing of agricultural products, attention is drawn to the need of restructuring, in order to increase efficiency, through a set of short, medium and long-term economic policy measures aimed at the labour market, education and industry.

The research is limited by the lack of indicators for longer periods of time, and for other countries, which would have allowed a comparative analysis. Moreover, a more complex picture would be provided by

including additional indicators, such as those that complement the factors analysed in this study as explanatory variables, but also related factors which explain certain results.

The paper sheds light on the significant role that the agricultural sector has had and will continue to have for Romania's economy, and on the fact that, although for almost a decade the organic agriculture's importance has increased, livestock production and agricultural services are still as important due to their effect on economic growth. Far from denying the importance of processing services and cereal production, their negative impact on growth is noticed. Therefore, at the decision-making level, these two activities should receive due attention, because processed agricultural products add greater value compared to the non-processed ones, and the scarcity of its processing industry places Romania at a disadvantage on the domestic and foreign market. The lack of processing industry stimulates the import and re-export, i.e., the export of primary agricultural products at low prices and their repurchase, in processed form, at much higher prices. We also draw attention to some changes in the agricultural labour market, following the conclusion that in Romania, salaried labour, but especially non-salaried labour, have a negative impact on growth; this requires a change in economic policy's strategy to allow the absorption of the labour force that has been replaced by technology in the agricultural sector. For the reasons above, the rethinking of the agriculture's employment policy is needed for a positive impact on growth.

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## ECOLOGICAL STUDY OF EARTHWORMS (OLIGOCHAETA: LUMBRICIDAE) DIVERSITY IN THE BOTANIC PARK OF TIMIȘOARA, ROMANIA

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### Abstract

*The geographic distribution of earthworms (Oligochaeta: Lumbricidae) has been only lowly studied in Romania, and for the urban diversity a few information is available. As a component of urban diversity, earthworms accomplish important roles in the soil ecosystems and in the global ecosystem, the largely known being the increase of soil fertility, the improvement of the physical-chemical properties of the soil and the management of wastes through vermicompostation. The goal of this study was to establish the species diversity of earthworms (Oligochaeta: Lumbricidae) in the Botanic Park of Timișoara, Romania, and to ecologically characterize this biodiversity through several specific ecological indices (abundance, dominance, constancy, Dzuba ecological signification, Sørensen and Jaccard similarity). Six earthworm species have been identified in the tehnosoil of the Botanic Park from Timișoara: Aporectodea rosea, Aporectodea caliginosa, Allolobophora chlorotica, Dendrodrilus rubidus, Lumbricus terrestris, Lumbricus rubellus.*

**Key words:** earthworms, diversity, urban, Oligochaeta: Lumbricidae.

### INTRODUCTION

The earthworms are encountered on Terra in all continents, excepting Antarctica (Phillips et al., 2019). These are organisms of Kingdom Animalia, Phylum Annelida (ringed worms), Class Clitellata (because of their anatomic structure named clitellum), Subclass Oligochaeta, Order Haplotaxida/Opisthopora (the genital male pores are open out of the body, posterior to the female genital pores), Family Lumbricidae (Rafinesque-Schmaltz, 1815).

In Romania, the common name of these organisms is "earthworms" ("râme" in Romanian).

There are approximately 700 earthworm species known all over the world, spread in various environments, excepting the areas with arctic and arid climates. In Europe live approximately 20 earthworm species widely spread, and in Romania there have been identified 80 species and subspecies (Szederjesi et al., 2019).

The geographic distribution of earthworms (Oligochaeta: Lumbricidae) has been only lowly studied in Romania (Pop & Pop, 2006;

Pop et al., 2012; Szederjesi et al., 2014; Csuzdi et al., 2018; Szederjesi et al., 2019), and for the urban diversity there are few information (Iordache et al., 2021).

As a component of urban diversity, earthworms accomplish important roles in the soil ecosystems and in the global ecosystem, those largely known being the increase of soil fertility, the improvement of the physical-chemical properties of the soil and the management of wastes (through vermicompostation).

The social ecology showed that human being cannot be separated by his native natural environment (Richardson et al., 2017; Lumber et al., 2017), and since the urban habitat seems to be the main living environment of the humans now and in the future, becomes more clear why every species of urban biodiversity is important in making the human habitat more understood, and, according to several opinions, more friendly as a indicator of wellness (Richardson et al., 2017; Li et al., 2019; Parsons et al., 2019).

The goal of this study was to establish the species diversity of earthworms (Oligochaeta: Lumbricidae) in the Botanic Park of Timisoara,

Romania, and to ecologically characterize this biodiversity through several specific ecological indices.

## MATERIALS AND METHODS

The researches was made in the Botanic Park of Timișoara ( $45^{\circ}45'18''\text{N}$ ,  $21^{\circ}13'28''\text{E}$ ) (Figure 1), located at north of river Bega, with 8,41 ha surface (Ciupa, 2010).

The Botanic Park of Timisoara is also known as Botanic Garden and it has been settled as an arboretum (dendrological park), established

between years 1986-1990 by the architect Silvia Grumeza. There have been planted here over 1650 plant species with various origins, like from other parks of Romania, private collections, or international exchange of seeds. In 1995 the park has been declared scientific reservation, aiming the protection of local and exotic flora (17, 18).

Across the park there have been established four sampling points (Figure 1) to study the earthworm species diversity. These sampling points have been established basing on casting occurrence.



Figure 1. The Botanic Park of Timisoara ( $45^{\circ}45'18''\text{N}$ ,  $21^{\circ}13'28''\text{E}$ ) (capture Google Maps): view with the sampling points

Earthworm sampling has been made using formalin solution combined with direct hand sampling. The taxonomic affiliation has been made on adult worms, the ecological feeding affiliation (epigeic, endogeic, anecic) has been made according to Bouché (1977). The values of abundance, dominance, constancy, index Dzuba have been calculated according to Gomoiu & Skolka (2001). The indicator

constancy has been calculated by relating the number of sampled plots where the species occurred to the total number of plots (Braun-Blanquet, 1932).

The indices Sørensen and Jaccard have been calculated based on species incidence in the sampled points according to Chao & Ricotta (2019).

## RESULTS AND DISCUSSIONS

Within the studied area of the park there have been identified six earthworm species. As habitat ecology, two of these species are epigeic, three are endogeic, and one of these is anecic (Table 1). The classification as feeding ecology showed three earthworm species detritivore and three geophage (Table 1).

Table 1. The earthworm species identified in the Botanic Park of Timisoara, Romania

No.	Species	Habitat ecology	Feeding ecology
1	<i>Aporrectodea rosea</i> (Savigny, 1826)	endogeic	geophage, microphage
2	<i>Aporrectodea caliginosa</i> (Savigny, 1826)	endogeic	geophage, microphage
3	<i>Allolobophora chlorotica</i> (Savigny, 1826)	endogeic	geophage, microphage
4	<i>Dendrodrilus rubidus</i> (Savigny, 1826)	epigeic	detritivore, mezophage
5	<i>Lumbricus terrestris</i> (Linnaeus, 1758)	anecic	detritivore, macrophage
6	<i>Lumbricus rubellus</i> (Hoffmeister, 1845)	epigeic, endogeic, anecic	detritivore, mezophage

In Table 2 is shown the distribution of earthworm species found in the four sampling points in the Botanic Park of Timisoara, with the mention that this is only for the adult individuals, the only ones that can be taxonomically identified.

Table 2. The distribution (species incidence) of earthworm species in the sampling points of the park

No.	Species	Sampling point 1	Sampling point 2	Sampling point 3	Sampling point 4
1	<i>Aporrectodea rosea</i>	x	x	x	x
2	<i>Aporrectodea caliginosa</i>	x		x	x
3	<i>Allolobophora chlorotica</i>		x		x
4	<i>Dendrodrilus rubidus</i>		x		
5	<i>Lumbricus terrestris</i>	x	x	x	x
6	<i>Lumbricus rubellus</i>	x	x		

In Table 3 there are presented the numerical and biomass abundance for the sampled earthworm species, both for adults and juveniles.

Table 3. The numerical and biomass abundance for the sampled earthworm species

No.	Species	Sampling point 1		Sampling point 2		Sampling point 3		Sampling point 4	
		Number of adults	Total biomass (adults+ juveniles) (g)	Number of adults	Total biomass (adults+ juveniles) (g)	Number of adults	Total biomass (adults+ juveniles) (g)	Number of adults	Total biomass (adults+ juveniles) (g)
1	<i>Aporrectodea rosea</i>	3	12 g	3	19 g	2	75 g	4	83 g
2	<i>Aporrectodea caliginosa</i>	1		-		1		3	
3	<i>Allolobophora chlorotica</i>	-		5		-		1	
4	<i>Dendrodrilus rubidus</i>	-		1		-		-	
5	<i>Lumbricus terrestris</i>	1		3		10		9	
6	<i>Lumbricus rubellus</i>	1		1		-		-	
		Total earthworms (adults+juveniles): 6		Total earthworm (adults+juveniles): 23		Total earthworms (adults+juveniles): 18		Total earthworms: (adults+juveniles) 41	

There was found that *Lumbricus terrestris* is the most abundant species in the studied urban ecosystem, both as number and biomass (Table 3), the weight of the adults ranging between 3 and 8 g.

The values of *dominance* (D) are listed in Table 4 and represent values more explanatory compared to the indicator *abundance* alone, and therefore more precise in establishing the numerically dominance ranking of the earthworm species within the studied urban ecosystem. There was thus found that four species are eudominant in the studied ecosystem: *Lumbricus terrestris*, *Aporrectodea rosea*, *Allolobophora chlorotica*, and *Aporrectodea caliginosa*, and *Dendrodrilus rubidus* is a recedent species encountered in a single sample, with one individual.

The values of the indicator *constancy* are shown in Table 5. There have been found three euconstant earthworm species, encountered in all samples: *Lumbricus terrestris*, *Aporrectodea rosea* and *Aporrectodea caliginosa*. *Dendrodrilus rubidus* is the only one accessory species.

Table 4. The values of the ecological indicator *dominance* (D) for the sampled earthworm species

No.	Species	Values of <i>dominance</i> (D)	Signification
1	<i>Aporrectodea rosea</i>	D sp. <i>Aporrectodea rosea</i> = 24.48%	eudominant species
2	<i>Aporrectodea caliginosa</i>	D sp. <i>Allolobophora chlorotica</i> = 10.20%	eudominant species
3	<i>Allolobophora chlorotica</i>	D sp. <i>Dendrodrilus rubidus</i> = 12.24%	eudominant species
4	<i>Dendrodrilus rubidus</i>	D sp. <i>Lumbricus terrestris</i> = 2.04%	recedent species
5	<i>Lumbricus terrestris</i>	D sp. <i>Lumbricus rubellus</i> = 46.93%	eudominant species
6	<i>Lumbricus rubellus</i>	D sp. <i>Lumbricus rubellus</i> = 4.08%	subdominant species

Table 5. The values of the ecological indicator *constancy* (C) for the sampled earthworm species

No.	Species	Values of <i>constancy</i> (C)	Signification
1	<i>Aporrectodea rosea</i>	C sp. <i>Aporrectodea rosea</i> = 100%	euconstant species
2	<i>Aporrectodea caliginosa</i>	C sp. <i>Allolobophora chlorotica</i> = 75%	euconstant species
3	<i>Allolobophora chlorotica</i>	C sp. <i>Dendrodrilus rubidus</i> = 50%	accessory species
4	<i>Dendrodrilus rubidus</i>	C sp. <i>Lumbricus terrestris</i> = 25%	accidental species
5	<i>Lumbricus terrestris</i>	C sp. <i>Lumbricus rubellus</i> = 100%	euconstant species
6	<i>Lumbricus rubellus</i>	C sp. <i>Lumbricus rubellus</i> = 50%	accessory species

The indicator *Dzuba* (W) was calculated to find out the relation existing between constancy (C) as a structural indicator and *dominance* (D) as a productive indicator, this combination offering a more accurate view about the ecological signification of the earthworm species found in the studied ecosystem as compared to the *dominance* (D) alone.

The ranking of these species according to the *Dzuba* indicator (W) is shown in Table 6.

Table 6. The values of the ecological indicator *Dzuba* (W) for the sampled earthworm species

No.	Species	Values of indicator <i>Dzuba</i> (W)	Signification
1	<i>Aporrectodea rosea</i>	W sp. <i>Aporrectodea rosea</i> = 24.48%	eudominant species
2	<i>Aporrectodea caliginosa</i>	W sp. <i>Allolobophora chlorotica</i> = 7.65%	dominant species
3	<i>Allolobophora chlorotica</i>	W sp. <i>Dendrodrilus rubidus</i> = 6.12%	dominant species
4	<i>Dendrodrilus rubidus</i>	W sp. <i>Lumbricus terrestris</i> = 0.51%	recedent species
5	<i>Lumbricus terrestris</i>	W sp. <i>Lumbricus rubellus</i> = 46.93%	eudominant species
6	<i>Lumbricus rubellus</i>	W sp. <i>Lumbricus rubellus</i> = 2.04%	subdominant species

To establish the values of the indicators Sørensen and Jaccard there have been considered the incidence (the presence or absence) of the earthworm species in the sampling areas.

There has been found a high Sørensen similarity between all pairs of sampling points, ranging between 50 - 100% (Table 7).

Table 7. Sørensen similarity (%) of the sampling points in the urban park: the values of the Sørensen indicator

	Sampling point 1	Sampling point 2	Sampling point 3	Sampling point 4
Sampling point 1	100%	0.66	0.85	0.75
Sampling point 2	66%	100%	0.50	0.66
Sampling point 3	85%	50%	100%	0.85
Sampling point 4	75%	66%	85%	100%

These values show that, as species composition, the sampling areas (selected on the basis of castings occurrence) are homogenous (over 50%), although the earthworms have been collected only from four sampling points related to the entire surface of the park.

The dendrogram of Sørensen similarity (Figure 2) shows that the sampling point 1 is that establishing the most numerous hierarchical clusters with the other sampling points, meaning the highest similarities as earthworm species composition, followed by the sampling point 4.

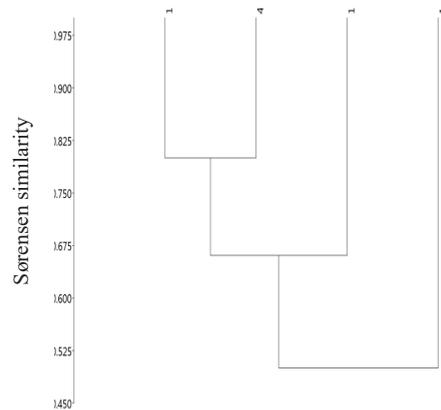


Figure 2. Dendrogram of Sørensen similarity (%) of the four sampling points across the urban park (the values range from 0 to 1, equivalent to 0 to 100% similarity)

In order to establish the relations of coenotic affinity between the earthworm species identified within the park, the *similarity Jaccard* has been calculated (Table 8).

A single pair of earthworm species was found to have 100% coenotic affinity: *Lumbricus terrestris* - *Aporrectodea rosea*.

Also, a single pair of earthworm species was found to have 0% coenotic affinity: *Aporrectodea caliginosa* - *Dendrodrilus rubidus*.

Two pairs of earthworm species had 75% coenotic affinity: *Aporrectodea rosea* - *Aporrectodea caliginosa* and *Lumbricus terrestris* - *Aporrectodea caliginosa*.

Because more than half of species pairs of earthworms (9 from 15) possess over 50% coenotic affinity, becomes clear the affinity of these species to co-inhabit the same biotope.

The cluster dendrogram of the Jaccard similarity (coenotic affinity) for the six

earthworm species identified within the park (noted with 1 to 6) (Figure 3) shows three very affine earthworm species: 1 - *Aporrectodea rosea*; 2 - *Aporrectodea caliginosa*; 3 - *Lumbricus terrestris*.

Two groups comprise species lowly affine: the group (1, 4, 6) and the group (1, 5, 6), which consist of the species (*Aporrectodea rosea*, *Dendrodriilus rubidus*, *Lumbricus rubellus*) and respectively (*Aporrectodea rosea*, *Lumbricus*

*terrestris*, *Lumbricus rubellus*), while the group formed by the species 1, 5 (*Aporrectodea rosea*, *Lumbricus terrestris*) presents 100% coenotic affinity. The species *Dendrodriilus rubidus* (4) is entirely separated from the others, with which it has no coenotic affinity (singleton). This species was also found to be rare in another urban park from Timișoara studied as earthworm species composition (Iordache et al., 2021).

Table 8. Diagram of Jaccard similarity (coenotic affinity - %) of the sampled earthworm species within the Botanic Park from Timisoara, Romania

Species	<i>Aporrectodea rosea</i>	<i>Aporrectodea caliginosa</i>	<i>Allolobophora chlorotica</i>	<i>Dendrodriilus rubidus</i>	<i>Lumbricus terrestris</i>	<i>Lumbricus rubellus</i>
<i>Aporrectodea rosea</i>		75%	50%	25%	100%	50%
<i>Aporrectodea caliginosa</i>			25%	0%	75%	25%
<i>Allolobophora chlorotica</i>				50%	50%	33.33%
<i>Dendrodriilus rubidus</i>					25%	50%
<i>Lumbricus terrestris</i>						50%
<i>Lumbricus rubellus</i>						

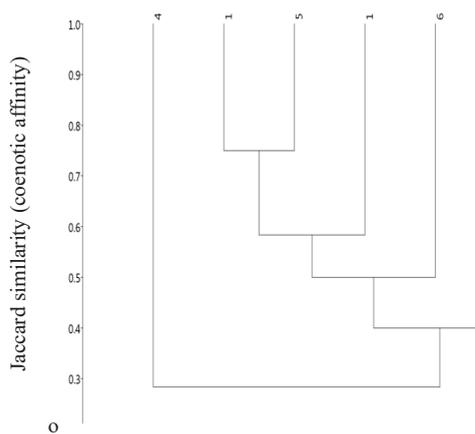


Figure 3. Dendrogram of Jaccard similarity (coenotic affinity): 1 - *Aporrectodea rosea*; 2 - *Aporrectodea caliginosa*; 3 - *Allolobophora chlorotica*; 4 - *Dendrodriilus rubidus*; 5 - *Lumbricus terrestris*; 6 - *Lumbricus rubellus*

## CONCLUSIONS

Six earthworm species have been identified in the tehno soil of the Botanic Park from Timisoara: *Aporrectodea rosea* (endogeic, geophage), *Aporrectodea caliginosa* (endogeic, geophage), *Allolobophora chlorotica* (endogeic, geophage), *Dendrodriilus rubidus* (epigeic,

detritivore), *Lumbricus terrestris* (anecic, detritivore), *Lumbricus rubellus* (epigeic, detritivore).

The species *Lumbricus terrestris* has been found to be the most abundant in the studied urban ecosystem, both numerically and as biomass.

Four earthworm species are numerically eudominant in the studied urban ecosystem: *Lumbricus terrestris*, *Aporrectodea rosea*, *Allolobophora chlorotica*, *Aporrectodea caliginosa*, and *Dendrodriilus rubidus* is a recedent species, encountered in one specimen in a single sampling surface.

Three earthworm species are euconstant, found in all sampling points: *Lumbricus terrestris*, *Aporrectodea rosea* and *Aporrectodea caliginosa*. *Dendrodriilus rubidus* is the only accessory species (one specimen in a single sampling surface).

As species composition, the studied perimeters are very homogenous, which is sustained by high values of the Sørensen similarity (9 from 15 sampling points show over 50% similarity). A single pair of earthworm species was found to have 100% coenotic affinity: *Lumbricus terrestris* - *Aporrectodea rosea*.

Also, a single pair of earthworm species was found to have 0% coenotic affinity:

*Aporrectodea caliginosa* - *Dendrodrilus rubidus*.

Because more than half of species pairs of earthworms (9 from 15) possess over 50% coenotic affinity, becomes clear the affinity of these species to co-inhabit the same biotope.

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## RESEARCH ON THE INCIDENCE OF MICROMYCETES ON WHEAT SEEDS DURING STORAGE IN VIEW DAMAGE CONTROL

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### Abstract

*Wheat is the main cereal crop in the EU- world production is about 582.7 million tons from 213.8 million ha. (FAOSTAT, 2020). Romania is a traditional grower and producer of wheat; therefore it is necessary to obtain a high quality of seed material. Storage fungi are among the major factors causing post-harvest deterioration of crop produce worldwide. FAO estimates that annually, through conditioning and storage, the percentage of losses reaches 6-10%. Three varieties of wheat seeds from crops in south-eastern Romania were analysed, in storage conditions. Measurements were aimed at determining the associated fungal load of wheat seeds and establishing their influence on quality indicators. The paper presents a study on the appearance and development of storage-specific micromycetes. The research was carried out on common wheat seeds from the warehouses of the National Administration of State Reserves and Special Issues. Wheat is stored during the cold season at an optimal level of temperature and humidity, to preserve quality. Seed testing for germination and incidence of was performed in the laboratory by the classic method of filter paper and PDA medium.*

**Key words:** wheat, isolation, storage, micromycetes.

### INTRODUCTION

Romania is, by tradition, an important producer of cereals, occupying on average an area of 2000-2500 thousand ha, cultivated with wheat, the annual grain harvest amounts to 15-20 million tons. An important part of the wheat harvest is consumed in rural households and is not traded. The rest of the wheat crop enters the commercial circuit, it is conditioned, stored and processed for internal consumption (Roman, et al., 2012). The seed occupies a special position in the measures that can be taken to increase the biological potential and phytosanitary status of plants. For this reason it is necessary for the seed to be a good genetic material, to have biological purity, high cultural value and to be free of pathogens (Placinta, 2007). Crop yields are dependent on the interactions of socio economic, biological, technological and ecological factors. (Kesho et al., 2020; Onwueme and Sinha 1999). The ideal daily temperature for wheat development varies from

20-25<sup>0</sup>C for germination, 16-20<sup>0</sup>C for good processing and 20-23<sup>0</sup>C for proper plant development (Beard et al., 2004). Specialist studies have shown that for some pathogens, seeds have an exclusive role in transmission and spread (Cristea & Berca, 2013).

The manifestation and the intensity of the damages produced by the micromycetes transmitted through the straw grain seed are an expression of the degree of its infestation, the virulence of the pathogen, the sensitivity of plants and environmental factors (Raicu et al., 1978).

The impact on the pathogenic fungus of wheat seed is necessary to determine its quality indicators. Research on the black spot wheat attack shows the special importance for both indices on the implications of the seed attack and the determination of the mycoflora associated with the production of this attack (Cristea & Berca, 2013).

Pathogenic fungi on wheat caryopsis in storage conditions were also observed corn seeds in the

same conditions (Dudoiu et al, 2016). Also, the fungi detected on corn seeds in storage conditions are fungi common to wheat seeds (Cristea et al., 2015; Cristea & Berca, 2013) but are also detected on the seeds of other species, being known as seed pots (Mardare et al., 2015; Manole & Cristea, 2015; Berca & Cristea, 2015; Pană, et al., 2014) affecting their qualitative indices (Matei et al., 2011; Tamba et al., 2010).

Stored cereals can have losses in both quantity and quality. Loss occurs during the storage of wheat grains after harvest due to biotic and abiotic factors. Wheat losses after harvest are estimated at about 8% of production. Several million colonies of storage fungi have been reported from a gram of dust collected from grain elevators and warehouses (Mathew, 2010).

The post-harvest loss of wheat cereals was found to be the largest during storage. The quality of cereals after harvest is influenced by a wide variety of biotic and abiotic factors and has been studied as a stored grain ecosystem. Stored grains can have losses in both quantity and quality. Losses occur when the grain is attacked by microorganisms and other organisms, including insects, mites, rodents and birds (Magan et al, 2003; Krasauskas et al., 2005; Zvicevičius et al., 2006; Neethirajan et al., 2007).

Cereal production varies from year to year and therefore cereals should be strategically stored from years of overproduction for use in the year under production. Cereals must also be stored because the point of production is not the point of consumption and the time of production is not the time of consumption. Stored grains can have losses in both quantity and quality. The quality of cereals after harvest is influenced by a wide variety of biotic and abiotic factors and has been studied as a stored grain ecosystem. Losses occur when the grain is attacked by microorganisms and other organisms, including insects, mites, rodents and birds. Loss of grain in quality can be in the form of depletion of seed viability, hardness, color, size and shape, grain weight and various biochemical parameters such as proteins, carbohydrates and vitamins in post-harvest

deposits (Kalsa et al., 2019; Kaminski & Christiaensen 2014; Uygun et al., 2005; FAOSTAT 2020; Stathers et al., 2013).

## MATERIALS AND METHODS

The research was carried out on common wheat seeds, 3 varieties: Balaton production 2019, Glosa production 2019, Dropia production 2015.

The wheat samples were stored in a cereal warehouse, in jumbo bags, placed on pallets; sampling from the bags for analysis was done with manual probes. The grain and microclimate temperature and humidity were determined as follow:

January-March: Microclimate: min T: -3° C and H: 35%; max T: 9°C and H: 88%; Product/grain: min T: 0°C and H: 11.4%; max T: 7.8°C and H: 13%;

April-May: Microclimate: min T: 1°C and H: 39%; max T: 18°C and H: 88%; Product/grain: min T: 4.4°C and H: 11.5%; max T: 15.3°C and H: 13%.

The storage rooms was natural ventilated.

The testing of the capacity of wheat seeds to produce plants in the field, by germination and the incidence of micromycetes was performed by incubating them on a drying substrate and agarized medium in optimal laboratory conditions, aims to assess their quality for sowing, marketing or long preservation duration.

Germination was determined according to the STAS 25°C method.

Three seeds of the same varieties were placed on each Petri dish (ø 10 cm) on an agarized medium (Hulea, 1969; Constantinescu, 1974), in several replicates. The water agar media was preferred due to its low nutrient composition which allows the fungi to grow but not its abundant sporulation.

This is an important step in order to be able to isolate each fungus from the Petri dish multitude of pathogens. The dishes were kept at room temperature (22-24°C) and normal light conditions. After 7 days there were performed macroscopic observations regarding the mycelia growth in Petri dishes, followed by optical microscope observations.

## RESULTS AND DISCUSSIONS

The associated mycoflora from wheat caryopsis in the deposits under analysis is represented by microorganisms such as micromycetes and yeasts. Our study was focused on the micromycetes analysis.

First of all we determined the germination of wheat caryopsis during the experimentation period (Table 1).

Table 1. The germination faculty of wheat caryopsis

Variety	Germination (%)	
	4 days	7 days
Balaton	90	95
Glosa	90	95
Drophia	85	90

The data in the Table 1, show that, after 7 days of observation, the germination of wheat caryopsis was not affected by any fungus varieties. At the Balaton production 2019 and Glosa production 2019 varieties, the germination was 95% and at the Drophia production 2015 the germination was determined at the 90%.



Figure 1. Germination of wheat caryopsis of Balaton after 7 days



Figure 2 Germination of Drophia wheat caryopsis after 7 days



Figure 3 Germination of Glosa wheat caryopsis after 7 days

The second step of our experiment was to isolate specific fungi from our samples. After incubation on agarized medium were isolated fungi that belong to predominant species of *Alternaria* spp., *Penicillium* spp., *Rhizopus* spp., and *Fusarium* spp. (Table 2). The micromycetes *Alternaria* spp., *Penicillium* spp., *Rhizopus* spp., and *Fusarium* spp. were also detected on the seeds of other plant species in Romania (Cozea et al., 2011; Zala et al., 2011; Mardare et al., 2014; Pana et al., 2014).

Table 2. Mycoflora detected on wheat caryopsis

Variety	The pathogen			
	<i>Alternaria</i> spp.	<i>Penicillium</i> spp.	<i>Fusarium</i> spp.	<i>Rhizopus</i> spp.
Balaton	+	+	-	-
Drophia	-	+	-	+
Glosa	+	-	+	-

The data in Table 2 show that the pathogens present on wheat caryopsis belonged to the species *Alternaria* spp., *Penicillium* spp., *Rhizopus* spp., *Fusarium* spp. The incidence of mycoflora show that *Alternaria* spp. was present on wheat caryopsis in the varieties Balaton and Glosa.

The micromycetes belonging to *Penicillium* spp were identified on Balaton and Drophia. The *Fusarium* spp fungi were present at the following to the Glosa variety. And the the micromycetes belonging to *Rhizopus* spp were identified on the Drophia variety.

## CONCLUSIONS

According to the results the following comments can be made on the mycoflora of wheat caryopsis. Germination of wheat seeds was not affected by the detected fungi. Our

research confirmed the presence of fungus associated with wheat seeds from the genera: *Alternaria*, *Fusarium*, *Penicillium*, *Rhizopus*. The most common isolated pathogenic species belong to the genus *Alternaria* spp and *Fusarium* spp and have colonized the wheat seeds of the studied varieties. The *Alternaria* spp fungus was identified on the Balton and Glosa varieties. The *Penicillium* spp fungus was identified on the Balatos and Dropia strains, and the *Fusarium* spp fungus was identified on the Glosa variety. The *Rhizopus* spp fungus were identified only on the Dropia variety.

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## CONTRIBUTIONS TO THE KNOWLEDGE OF COLEOPTERS SPECIES EXISTING IN SOME WALNUT ORCHARDS

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### Abstract

*During the research period, at the Sarca stationary belonging to SCDP Iasi, were studied two walnut plantations, one represented a plantation in which was developed a technology that included the application of chemical treatments, and the other stationary was also represented by a walnut orchard in which no were applied methods of protection against diseases and pests. In order to establish the structure and the ecological parameters representative of the coleopters entomofauna from the two plantations, were placed six soil traps type Barber, which had as fixing liquid a salt solution (NaCl) in a concentration of 25%, but the method of tapping was also used. The material was harvested periodically, at intervals between 7 and 14 days, and at each collection the biological material from the traps was transferred from the trap to a gauze cloth, was labeled, and the fixing liquid was filled or replaced according to case. Larger, significant differences appear from one stationary to another, which indicates that the technology with the treatment scheme applied in vegetation has a great influence on the entomofauna in general.*

**Key words:** Coleoptera, walnut plantations, useful entomofauna.

### INTRODUCTION

Following the profound social and political transformations in the life of the country, agriculture registered significant successes, the level of production increased faster than the demographic growth.

The changes that took place in Romanian agriculture therefore demonstrated the possibility of rapidly increasing food resources. Among the factors that contributed to obtaining higher and higher yields was the increasingly efficient protection of crops.

The study of coleopterological associations in assessing the degree of ecosystem alteration and in assessing climate change (Tălmăciu et al., 2016) is currently widely used and is justified by the fact that the Order Coleoptera has the following advantages prone to these studies: it is the largest and most widespread group in the entire animal kingdom; is represented by species in all types of terrestrial and aquatic habitats (except seas and oceans); the species covers all heterotrophic trophic categories in an ecosystem.

Based on their numerous presence and frequency in ecosystems, various mathematical models of the dynamics of some animals of

economic interest could be constructed, principles and ways of forecasting and regulating the various dangerous pests in agriculture and horticulture could be developed.

The impact of pests on fruit plantations is the result of the interaction between a particular host /parasite system and local environmental and culture conditions (Baicu & Săvescu, 1978). Due to the expansion of organic farming, there have been changes in the spectrum of diseases and pests in agricultural crops, compared to a conventional system crop, where chemical treatments are applied.

### MATERIALS AND METHODS

The collection of the biological material was done with the help of two methods, namely: were placed soil traps type Barber, but the method of threading (mowing with entomological net) was also used.

The method of traps type Barber was represented by 6 traps arranged in a row on the row of trees, and the samples were collected from June to September.

Barber traps are the plastic boxes with a volume of 800 ml that are placed into ground level. The boxes were buried carefully, so that

the edge of the trap was perfectly level with the ground, and the insects could easily penetrate (Tălmaciu et al., 2010).

In Barber soil traps, was used a 25% concentration salt solution as the fixing liquid. The fixing fluid has a great influence on the effectiveness of the traps and must have good preservative qualities to prevent the maceration of the captured individuals.

By placing at least 6 traps, all species of Coleoptera order can be collected to establish dominance in a biotope.

At each collection, the contents of each box were placed on a sieve lined with a gauze bandage to separate the insects from the fixative. The gauze with each sample was placed in labeled jars. After each collection, the trap was re-inserted into the soil and the fixing fluid was replaced. With the help of the entomological net, mowing is performed on the surface of the soil or plants (sewing method). The entomological net is a device that is composed of a metal frame in the shape of a circle or triangle with a diameter or side of 30 cm, which has attached a frustoconical mesh and a rod with a length of about 0.80 m.

During the research period, several threads were made in the two stations represented by the ecological walnut plantation and the conventional walnut orchard.

The material thus collected was placed in jars in which a cotton swab soaked in chloroform was introduced for the almost instantaneous killing of the collected entomofauna.

The biological material thus collected using the 2 methods was brought to the laboratory, then cleaned of plant debris and then identified and inventoried, down to the species level.

The collection of the material by these methods was done on the following the dates: 22.05.2019; 18.06.2019; 06.07.2019; 27.07.2019; 07.08.2019; 27.08.2019.

## RESULTS AND DISCUSSIONS

By applying the Barber trap method in the ecological walnut orchard (Table 1), following the 6 harvesting from the summer of 2019, were captured 123 specimens belonging to a number of 34 species of coleopters (Panin, 1951; Reitter, 1908;)

Table 1. The coleopteran fauna from the ecological walnut plantation collected by the soil trap type Barber method in the Sârca stationary in 2019

Order	Name of species	No of harvesting						Total samples
		I	II	III	IV	V	VI	
Coleoptera	1. <i>Harpalus pubescens</i> Müll	10	3	9	17			39
	2. <i>Harpalus tenebrosus</i> Dej	3	16	9	2	8	1	39
	3. <i>Harpalus calceatus</i> Duft.	11	1	5	10	2	9	37
	4. <i>Harpalus distinguendus</i>	11	1	12	2	3	3	32
	5. <i>Hister quadrimaculatus</i> L.	4	1	4			2	11
	6. <i>Pterostichus cupreus</i> L.	1		8	1			10
	7. <i>Dermestes lanarius</i> Illig.	1	2			2	3	8
	8. <i>Aphthona euphorbiae</i> Sch		2	4		1	1	8
	9. <i>Harpalus aeneus</i>		1	2	1	1	1	6
	10. <i>Agriotes ustulatus</i> Schall		1	3		1	1	6
	11. <i>Metabletus truncatulus</i> L		1	4		1		6
	12. <i>Anisodactylus binotatus</i> F			1		1	4	6
	13. <i>Ottiorhynchus kollari</i> Gyll.	1		2			2	5
	14. <i>Ottiorhynchus fuscipes</i> O.		2	2		1		5
	15. <i>Coccinella 7 punctata</i> L.		2				3	5
	16. <i>Opatrum sabulosum</i> L.	2		1		1		4
	17. <i>Rhizophagus politus</i> Hel.	1		1			1	3
	18. <i>Amara aenea</i> Deeg		1			1	1	3
	19. <i>Cyaniris cianea</i> F		1			2		3
	20. <i>Anthicus humeralis</i> Geb.		2				1	3
	21. <i>Anthicus floralis</i> L	1		1			1	3
	22. <i>Psylliodes affinis</i> Payk			1			2	3
	23. <i>Pteryngium crenatum</i> Gyll	1		1				2
	24. <i>Ceuthorynchus obsoletus</i>		2					2
	25. <i>Apion apricans</i> Herbst		2					2

	26. <i>Apion virens</i> Herbst		1	1				2
	27. <i>Nebria brevicollis</i> F			1		1		2
	28. <i>Tachyusa constricta</i> Erich			2				2
	29. <i>Oxypora vittata</i> Mär			1			1	2
	30. <i>Tachyporus abdominalis</i> F			2				2
	31. <i>Pentodom idiota</i>			1		1		2
	32. <i>Quedius cinctus</i> Payk		1					1
	33. <i>Chrysomela marginata</i> L		1					1
	34. <i>Longitarsus gracilis</i> Kuts.			1				1
<b>Total samples</b>		47	44	78	33	27	37	266

In 2019 the species with largest number of sample collected were: *Harpalus pubescens* and *Harpalus tenebrosus* (39 samples), *Harpalus calceatus* (37 samples), *Harpalus distinguendus* (32 samples), *Hister quadrimaculatus* (11 samples), *Pterostichus cupreus* (10 samples), *Dermestes lanarius* and *Aphthona euphorbiae* (8 samples); *Harpalus aeneus*, *Agriotes ustulatus*, *Metabletus truncatulus*, *Anisodactylus binotus* (6 samples); *Otiorhynchus kollari*, *Otiorhynchus fuscipes*, *Coccinella 7 punctata* (5 samples). In the case of the following species, the number of specimens collected is between 1 and 5: *Opatrum sabulosum*, *Rhizophagus politus*, *Amara aenea*,

*Cyaniris cianea*, *Anthicus humeralis*, *Anthicus floralis*, *Psylliodes affinis*, *Pteryngium crenatum*, *Ceuthorynchus obsoletus*, *Apion apricans*, *Apion virens*, *Nebria brevicollis*, *Tachyusa constricta*, *Oxypora vittata*, *Tachyporus abdominalis*, *Pentodom idiota*, *Quedius cinctus*, *Chrysomela marginata* and *Longitarsus gracilis*.

In the conventional (chemical treated) walnut orchard (Table 2), following the 6 harvesting from the summer of 2019, were captured 118 specimens belonging to a number of 6 species. (Gaetan, 1990; Panin, 1951; Reitter, 1908; Rogojanu & Perju, 1979).

Table 2. The coleopteran fauna from the conventional walnut orchard collected by the soil trap type Barber method in the Sârca stationary in 2019

Order	Name of species	No of harvesting						Total samples
		I	II	III	IV	V	VI	
Coleoptera	1. <i>Opatrum sabulosum</i> L.	1	1	21		1	3	27
	2. <i>Harpalus aeneus</i>			3	6		8	17
	3. <i>Coccinella 7 punctata</i> L.			8			4	12
	4. <i>Harpalus pubescens</i> Müll		3	7	2			12
	5. <i>Aphthona euphorbiae</i> Sch			4		1	1	6
	6. <i>Amara crenata</i> Deeg		1		4		1	6
	7. <i>Harpalus azureus</i> F.		2	2		1		5
	8. <i>Harpalus calceatus</i> Duft.		4	1				5
	9. <i>Anthicus floralis</i> L			1	1		2	4
	10. <i>Chrysomela marginata</i> L			1			2	3
	11. <i>Dermestes lanarius</i> Illig.			1		2		3
	12. <i>Microlestes maurus</i> Sturm				1		2	3
	13. <i>Ophonus sabulicola</i> Panz				1	2		3
	14. <i>Pteryngium crenatum</i> Gyll			2			1	3
	15. <i>Nebria brevicollis</i> F			1		1		2
	16. <i>Oxypora vittata</i> Mär	1	1					2
	17. <i>Pentodom idiota</i>			1		1		2
	18. <i>Apion virens</i> Herbst			1				1
	19. <i>Otiorhynchus ovatus</i> L.	1						1
	20. <i>Pterostichus nigrita</i> L.	1						1
<b>Total samples</b>		4	12	54	15	9	24	118

In 2019 the species with largest number of sample collected were: *Opatrum sabulosum*, (27 samples), *Harpalus aeneus* (17 samples),

*Coccinella 7 punctata* and *Harpalus pubescens* (12 samples), *Aphthona euphorbiae* and *Amara crenata* (6 samples); *Harpalus azureus* and

*Harpalus calceatus* (5 samples); *Anthicus floralis* (4 samples), *Chrysomela marginata*, *Dermestes lanarius*, *Microlestes maurus*, *Ophonus sabulicola* and *Pteryngium crenatum* (3 samples), *Nebria brevicollis*, *Oxypora vittata* and *Pentodom idiota* (2 samples) and *Apion virens*, *Otiorynchus ovatus*, *Pterostichus nigrita* (2 samples).

The situation of the collections with beating method in the walnut ecological orchards, in 2019 was as follows:

At the first collection on 22.05.2019, we identified: 16 species of coleopters with 50 samples, with the highest abundance the species were registered: *Meloe variegatus*, *Longitarsus anchusae*, *Mordella maculosa*, *Apion nigritarse*, *Apion fuscirostre*, *Longitarsus anchusae* and *Mordella maculosa*.

At the second collection on 18.06.2019, there were registered: 13 species of coleopters with

38 samples, with the highest abundance the species were registered: *Ceutorhynchus napi*, *Apion fuscirostre*, *Ceutorhynchus troglodytes*, *Apion fuscirostre* and *Meligetes flavipes*.

At the third collection dated 06.07.2019, were registered only 2 species with 2 samples, and these were: *Cantharis lateralis* and *Meligetes flavipes*.

At the fourth collection on 27.07.2019, we were identified: 8 species of coleopters with 19 samples, the species with the highest abundance were registered: *Ceutorhynchus napi*, *Apion nigritarse*, *Cantharis lateralis* and *Longitarsus ochroleucus*.

At the fifth collection on 05.07.2019, we were identified: 5 species of coleopters with 13 samples, these species registered were: *Meloe variegatus*, *Ceutorhynchus napi*, *Apion nigritarse*, *Malachius bipustulatus* and *Apion apricans*.

Table 3. Entomofauna from the ecological apple orchard collected by the sewing method in the stationary Sârca in 2019

Order	Name of species	No of harvesting						Total samples
		I	II	III	IV	V	VI	
Coleoptera	<i>Mordellistena parvula</i> Gyll	2					1	3
	<i>Meloe variegatus</i> Don	5				1		6
	<i>Longitarsus anchusae</i> Payk	5						5
	<i>Hippodamia variegata</i> obverse punct. Goeze	3	2					5
	<i>Mordella maculosa</i> Nae	5	2					7
	<i>Ceutorhynchus napi</i> Gyll.	1	3		4	7		8
	<i>Apion vicinum</i> Kirby	3			2			5
	<i>Apion nigritarse</i> Kirby	5	2		3	1		11
	<i>Apion fuscirostre</i> Fa	6	3					9
	<i>Haltica oleracea</i> Linnaeus	1						1
	<i>Longitarsus ochroleucus</i> Mar		1				2	3
	<i>Longitarsus anchusae</i> Payk	5						5
	<i>Hippodamia variegata</i> obverse punct. Goeze	3					8	11
	<i>Cantharis lateralis</i> L	2	2	1	3		1	9
	<i>Apion vicinum</i> Kirby	3						3
	<i>Ceutorhynchus troglodytes</i> Germar		5					5
	<i>Apion ebenimum</i>		2		1			3
	<i>Apion fuscirostre</i> F		4					4
	<i>Meligetes flavipes</i> Sturm		9	1				10
	<i>Baris artemisiae</i> Herbst		2		1			3
	<i>Longitarsus ochroleucus</i>		1		3		1	5
	<i>Aphthona cyparissiae</i> Koch				2			2
	<i>Malachius bipustulatus</i> L.					3		3
	<i>Rhyncolus truncorum</i> Ger						1	1
	<i>Apion apricans</i> Herbst					1		1
	<i>Apion atomarium</i> Kirby						1	1
	<i>Mordella aculeata</i> L						3	3
<i>Ootyplus globulosus</i> Walt						1	1	
<i>Psylliodes affinis</i> Payk						1	1	
Total samples		50	38	2	19	13	20	142

At the sixth collection on 27.08.2019, we were identified: 10 species of coleopters with 20 samples, the species with the highest abundance were registered: *Mordellistena parvula*, *Longitarsus ochroleucus*, *Hippodamia variegata obverse punct.*, *Cantharis lateralis*, *Longitarsus ochroleucus*, *Rhyncolus truncorum*, *Apion atomarium*, *Mordela aculeate*, *Ootypus globulosus* and *Psylliodes affinis*.

The situation of the collections with sewing method in the walnut ecological orchards, in 2019 was as follows:

At the first collection on 22.05.2019, we identified: 16 species of coleopters with 50 samples, with the highest abundance the species were registered: *Baris analis*, *Baris lapidii*, *Cantharis plaudosa*.

At the second collection on 18.06.2019, there were registered: 7 species of coleopters with 17 samples, with the species registered were: two species of genus *Subcoccinella*, *Epuraea pygmaea*, *Malachius bipustulatus*, *Longitarsus anchusae*, *Ceutorhynchus napi* and *Epuraea pygmaea*.

At the third collection dated 06.07.2019, were not registered specoes of coleopters.

At the fourth collection on 27.07.2019, we were identified: 4 species of coleopters with 19 samples, the species with the highest abundance registered were: *Apion urticarium*, *Longitarsus anchusae*, *Phyllotreta atra*, *Ceutorhynchus napi*, *Apion apricans* and *Agriotes* sp.

Table 4. Entomofauna from the treated apple orchard collected by the sewing method in the stationary Sârca in 2019

Order	Name of species	No of harvesting						Total samples
		I	II	III	IV	V	VI	
Coleoptera	<i>Baris analis</i> Olivier	6						6
	<i>Baris lapidii</i> Germar	5						5
	<i>Cantharis plaudosa</i> Fa	2						2
	<i>Subcoccinella</i> 24punct. var. <i>saponariae</i> L.		2					2
	<i>Subcoccinella</i> 24punct. var. <i>4 notata</i> L.		2					2
	<i>Epuraea pygmaea</i> Gyll		3					3
	<i>Malachius bipustulatus</i> L		2					2
	<i>Longitarsus anchusae</i> Payk		3		4			7
	<i>Phyllotreta atra</i> Fabricius				2	3		5
	<i>Ceutorhynchus napi</i> Gyll.		2		3			5
	<i>Apion apricans</i> Herbst				3	6		9
	<i>Apion urticarium</i> Herbst				7	2		9
	<i>Agriotes</i> sp.				1			1
	<i>Apion vicinum</i> Kirby						8	8
	<i>Apion nigrirtarse</i> Kirby						16	16
	<i>Apion laevigatus</i> Kirby						5	5
	<i>Rhinomias forticornis</i> Boh					14		14
	<i>Monotoma picipes</i> Herbst						10	10
<i>Coccinella 7 punctata</i> L						1	1	
<i>Lixus ascanii</i> L						5	5	
Total		13	17	0	19	25	45	117

At the fifth collection on 05.07.2019, we were identified: 4 species of coleopters with 13 samples, these species registered were: *Rhinomias forticornis*, *Apion urticarium*, *Phyllotreta atra* and *Apion apricans*.

At the sixth collection on 27.08.2019, we were identified: 6 species of coleopters with 45 samples, the species with the highest abundance were registered: *Apion vicinum*,

*Apion nigrirtarse*, *Apion laevigatus*, *Monotoma picipes*, *Coccinella 7 punctata* and *Lixus ascanii*.

## CONCLUSIONS

In the environmental conditions that influenced the walnut plantations cultivated in the two technological systems (conventional and

ecological) during 2019, with the help of the two collection methods (threading and Barber type soil traps) following the 6 harvests have established the following:

With the help of the Barber method, most species of beetles were collected from the ecological walnut orchard, and the number of specimens also collected in this lot was 266.

In the chemically treated experimental group, the number of coleoptera species was 20, and the total specimen was 118.

By applying the second method, sewing method with the help of the entomological file, we recorded that the largest number of specimens collected in the ecological group and was represented by 29 species, and in the chemically treated group the number of copies was 117 ce they belonged to a number of 20 species.

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## MORPHO-ANATOMICAL CHARACTERS OF THE INVASIVE SPECIE *Iva xanthiifolia* Nutt.

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### Abstract

*Iva xanthiifolia* is an adventive species belong to the Asteraceae family, originated from North America. It is toxic specie, abundantly near the roads and rail ways in some Romanian areas like Dobrogea, Moldova and Transilvania and it can become a weed in the field crops. It is annual plant having 1 - 2,5 m height. The leaves are opposed in the lower part of the stem and alternated in the upper part, long-petiolate, ovate, double serrate, trinervate and scabrous. Morpho-anatomical analyses were made at the stems and leaves collected from the plants meet on the salt soil in the North-East of Dobrogea region, in the Plopol village of the Tulcea county. The transversal sections were made in the stem, petiole and lamina of leaves. The microscopic observations were made in both epidermis of the lamina leaf. The secretory canals were identified in the cortex of the stem. The vascular bundles from the stem and petiole are bicollateral. The non-glandular and secretory pluricellular hairs were observed in both epidermis of the leaves. The leaves are amphistomatic and the stomates were identified in upper and lower epidermis. The stomata are anomocytic type. The mesophyll of the leaf is bifacial.

**Key words:** *Iva xanthiifolia*, anatomy, toxic specie, annual plant.

### INTRODUCTION

The Genus *Iva* L. belongs to the Asteraceae family, with 12 species native to North America (The Plant List, 2013).

*Iva xanthiifolia* Nutt., Synonym *Cyclachaena xanthiifolia* (Nutt.) Fresen. is native to North America, being later introduced and naturalized in Central Europe and Asia, respectively (Tutin et al., 1980; Pruski, 2005).

In Romania, *Iva xanthiifolia* is on the list of invasive species, being found locally abundant on saline soils, in Dobrogea, Moldova, Transylvania regions and western country (Oradea), along roads, railways, together with *Ambrosia* species and it is representing a potential spread in crops (Anastasiu & Negrean, 2005; Ciocărlan, 2009; Niculescu et al., 2013).

The species has also been reported in Europe, in Croatia and Serbia and in the southeastern part of Hungary where it has been found along the roads and also in field crops, such as corn, sunflower, soybeans and sugar beets (Marisavljević et al., 2007; Konstantinovic et al., 2006).

In China, it was first found in the Dayingzi commune region of Liaoning province in 1981 year and in other areas such as Shenyang in 1982 (Xu et al., 2012, quoted by Guohua et al., 2017)

Today, climate change is making its mark on agriculture and imposing appropriate, sustainable agricultural practices in a sector that needs to provide food for the world's growing population (Delian et al., 2019). In addition, global climate change, as well as globalization have a negative impact on biodiversity, land use, and human well-being, including the spread of alien species invasions in Europe as well (Schindler et al., 2015), such as the *Iva xanthiifolia* Nutt. species (burweed marshelder), characterized by its allergenic pollen (Follak et al., 2013).

Invasion of various species is one of the main causes of the decline of biodiversity (Juhász & Juhász, 2006; Szatmari, 2012; Oteves et al., 2014).

In addition to its potential for spreading in the crops, *Iva xanthiifolia* is a toxic plant during its flowering, causing allergic reactions to humans, like many other plants of Asteraceae family,

being a major problem in recent years (Rodinkova et al., 2018; Pietrzyk et al., 2019). *Iva xanthiifolia* has a strong adaptability, rapid growth, competitiveness and reproductive capacity (Hodi & Torma, 2002).

One of the propagation sources of the *Iva xanthiifolia* species could be the reflected light of industrial waste, which changes the anatomical structure of the leaf and the photosynthetic capacity (Zhang et al., 2011).

Studies performed with aqueous extracts obtained from different organs of *Iva xanthiifolia* highlight the allelopathic effect with some agricultural species (Hunyadi et al., 1998; Hodişan, 2009).

The species from the Asteraceae family frequently present different types of hairs on the stems and leaves and secretory canals in the internal structure within with taxonomic, ecological and economic importance (Metcalf & Chalk, 1979; Zubaidah & Lateef, 2017; Taşar et al., 2018).

At the Asteraceae species, the anatomical diversity of vegetative organs can be observed at the level of vascular bundles, epidermis, type and distribution of stomata and leaf mesophile (Metcalf & Chalk, 1957; Millan et al., 2006; Zhang et al., 2011).

To understand the biological characteristics of *Iva xanthiifolia*, as support to prevent its spreading in the crops, it is necessary to better know the morpho-anatomical properties of the plant.

## MATERIALS AND METHODS

The stems and leaves of *Iva xanthiifolia* were harvested in early of September, from plants found on saline soils in Plopu village in Tulcea county, Romania, located at N 45°01'36.3"; E 29°07'38.1", near the Danube Delta.

The climate is dry and arid specific to the steppe zone with less than 400 mm per year rainfall and 12.2°C the average temperature.

The morphological proprieties (plant highest, leaves disposal, type of stem and inflorescence, branching type) were made in the field.

Cross-sections were made in the stems and leaves using the blade and there were clarified with Chloral-hydrate coloured, with the Carmine-Alaunate and Iodine Green.

The anatomical structures were analysed using the optical microscope Leica DM1000 LED, Camera video Leica DFC295, the Stereomicroscope Leica S8 APO, belonging to the Laboratory of Microscopy and Plant Anatomy of the University of Agronomic Sciences and Veterinary Medicine of Bucharest.

## RESULTS AND DISCUSSIONS

### *Plant morphology*

At the beginning of September, the growth stage of the *Iva xanthiifolia* plants were at the end of flowering - beginning of fruiting. The plants grow in compact groups, reaching heights of 1.5-2.5 m (Figure 1).

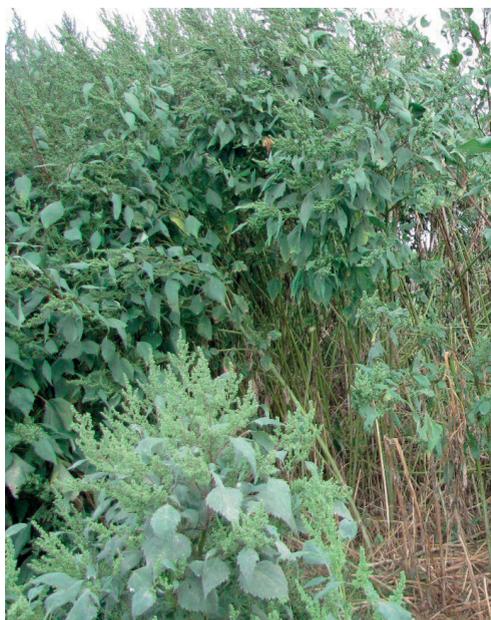


Figure 1. *Iva xanthiifolia* - Plopu, Tulcea

Leaves are opposed in the lower part of the stem and alternated in the upper part, long-petiolate, ovate, double serrate, trinervate and scabrous, often hairy on the lower side, with short and rough hairs.

The anthodia are small, nutant, numerous, paniculate grouped, with unisexual flowers (Figure 2). The male flowers are inside of the anthodium and the female flowers are outside, accompanied by an involucre formed by 5 lathe-ovate hypsophylls, almost round, rough hairy with sessile glands having below a female

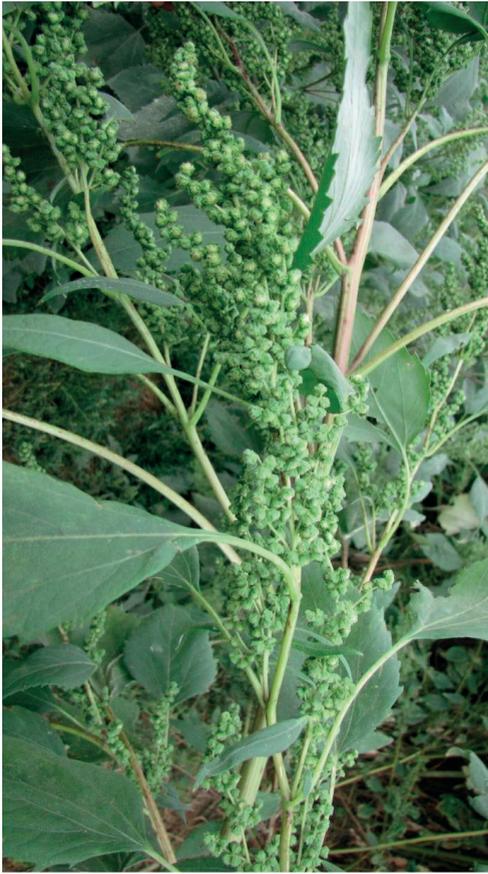


Figure 2. *Iva xanthiifolia* - inflorescence, Plopuł, Tulcea

flower formed by a single obovate ovary with two stigmas. The male flowers are tubular, 5-toothed, yellow-green, longer and visible until maturity, with sessile glands on the outside, consisting of 5 stamens and a stunted stalk, without ovary. At maturity, the marginal fruits surround the male flowers (Săvulescu, 1964; Ciocărlan, 2009).

#### Plant anatomy

Few anatomical studies on *Iva xanthiifolia* have been found in the literature.

The anatomy of the *Iva xanthiifolia* species largely corresponds to the family Asteraceae, according to data from the literature (Andrei, 1978; Sirbu C. & Paraschiv Nicoleta-Luminița, 2005; Cristea, 2014).

#### Anatomy of stem

The contour of the cross section is circular, with the thin edges (Figure 3).

The epidermis is unstratified, with isodiametric cells covered by a thick cuticle. The non-glandular, multicellular and uniseriate hairs (3-4 cells) were very rarely observed on the epidermis. Stomata were also rarely observed in the epidermis.

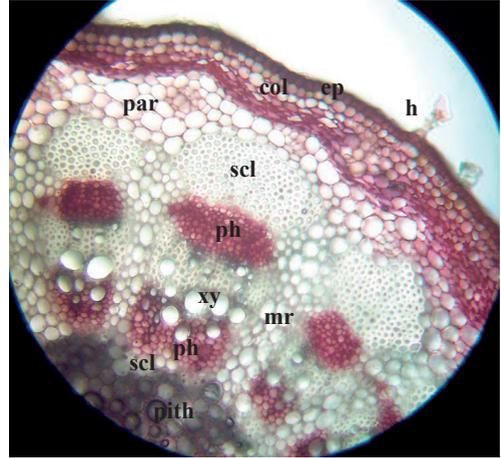


Figure 3. Cross section in the stem *Iva xanthiifolia*: ep - epidermis; col - collenchyma; par - parenchyma; scl - sclerenchyma; ph - phloem; xy - xylem; mr - medullary rays; pith; h - hair

There is a thin cortex, consisting of angular collenchyma, with 3-5 rows of cells and assimilating parenchyma consisting of 2-3 rows of cells. Endoderm is not obvious. At the level of the internal cortex, in the interfascicular area, secretory canals were observed, according to the data from the literature (Metcalf & Chalk, 1979) (Figure 4).

The vascular bundles are bicollateral type and cyclically arranged with external and internal phloem, between which is the xylem (Figure 5). This type of bundles is reported in the literature for some Asteraceae species (Andrei, 1978; Cristea, 2014; Tașar et al., 2018).

In the internal structure of the stem 13-15 vascular bundles were observed. The vascular bundles are bordered by sclerenchyma. The sclerenchyma is thicker at the periphery of the external phloem.

The phloem consists of phloem vessels, annex cells and few parenchyma cells.

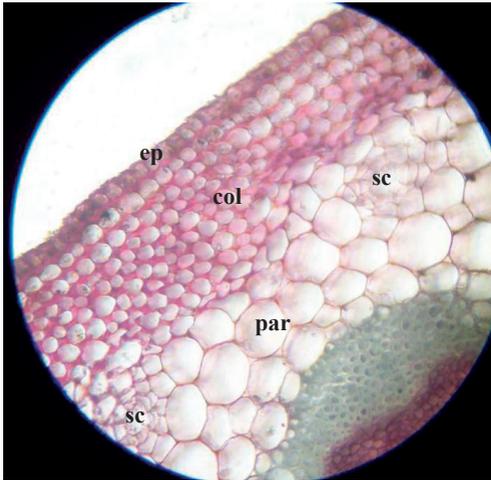


Figure 4. Cross section in the stem  
*Iva xanthiifolia*: ep - epidermis; col - collenchyma;  
par - parenchyma; sc - secretory canal

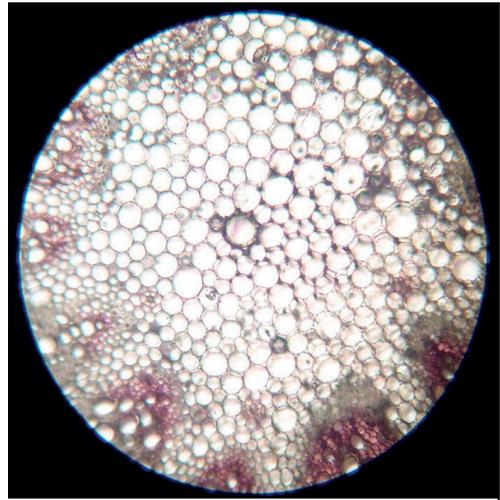


Figure 6. Cross section in the stem - pith  
*Iva xanthiifolia*

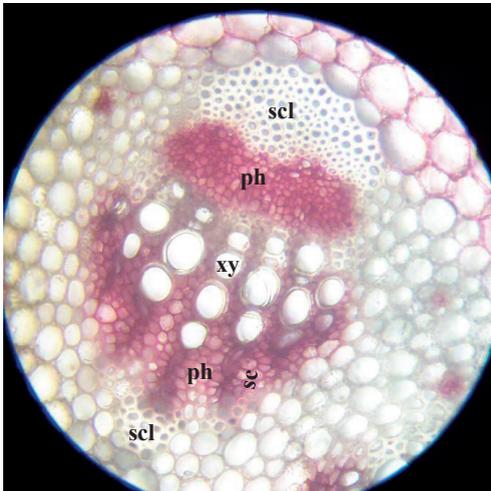


Figure 5. Cross section in the stem  
*Iva xanthiifolia*: xy - xylem, ph - phloem,  
sc - secretory canal, scl - sclerenchyma

The xylem consists of xylem vessels arranged in radial rows, xylem parenchyma and xylem fibres. Between the xylem vessels there are libriform elements.

At the level of internal phloem, secretory canals were observed.

The pith is parenchymatic of the meatic type (Figure 6).

At the periphery of the pith, under the vascular bundles, interfascicular, there were secretory canals observed. The prismatic mineral crystals have also been identified.

#### *Anatomy of the leaf*

The epidermises, seen from the front, are made up of cells with strongly corrugated walls (Figures 7 and 8).

The stomates are of the anomocytic type and are present in both epidermises, the leaf being amphistomatic.

In both epidermises, the non-glandular and glandular hairs have been observed. In the abaxial epidermis, the hairs are multicellular and more frequently.

According to the results of some studies, two types of glandular hairs have been identified: long hairs, multicellular uniseriate, with apical secretory cell and short hairs, multicellular and biseriate (Lana et al., 2007).

On the adaxial epidermis the observed hairs were shorter and straighter and on the abaxial epidermis there were longer and slightly curved hairs.

From an anatomical point of view, the leaf lamina has a dorsi-ventral structure, bifacial lamina has a palisade tissue under the adaxial epidermis and spongy tissue under the abaxial epidermis (Figure 9). The palisade tissue consists of a single row of elongated cells, with a high content of chloroplasts and the spongy tissue consists of 3-4 rows of cells, ovoid, with intercellular spaces and a lower content of chloroplasts.

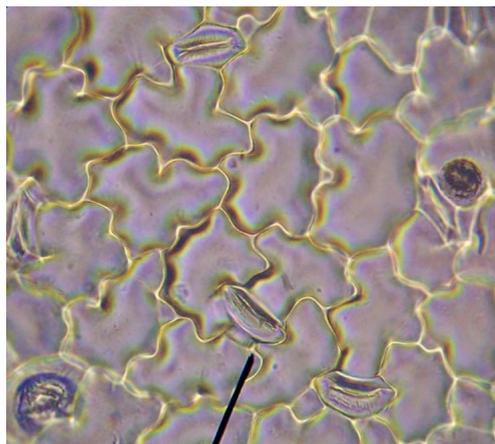


Figure 7. Adaxial epidermis of the leaf  
*Iva xanthiifolia*

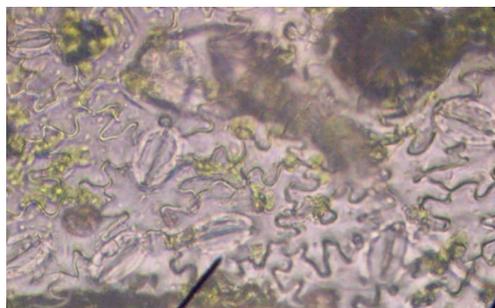


Figure 8. Abaxial epidermis of the leaf  
*Iva xanthiifolia*

Collateral-closed vascular bundles are present in the mesophyll of the leaf. Raphide-shaped mineral crystals were also observed in the mesophyll.

The median rib is much more prominent in the lower part of the lamina.

Under the epidermis is the collenchyma with 2-3 rows of cells. In the parenchyma there are 3 collateral-closed vascular bundles with the median bundle larger than the laterals, with xylem on the outside and phloem on the inside, being surrounded by a parenchyma sheath. The hairs are longer and curved on the abaxial epidermis of the rib and are shorter and straighter on the adaxial epidermis.

#### *Petiole structure*

The cross-section shape of the petiole is round with two adaxial wings. The epidermis is single layered, with small isodiametric cells, with thick external walls. From place to place, stomata are present.

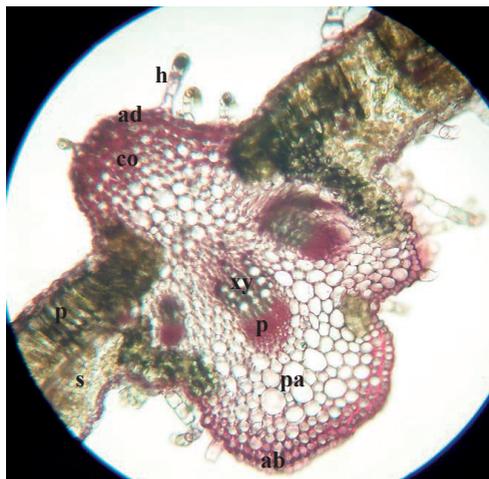


Figure 9. Transverse sections of the lamina  
*Iva xanthiifolia*: ade - adaxial epidermis, abe - abaxial epidermis, pt - palisade tissue, st - spongy tissue, col - collenchyma, par - parenchyma, xy - xylem, ph - phloem, h - hairs

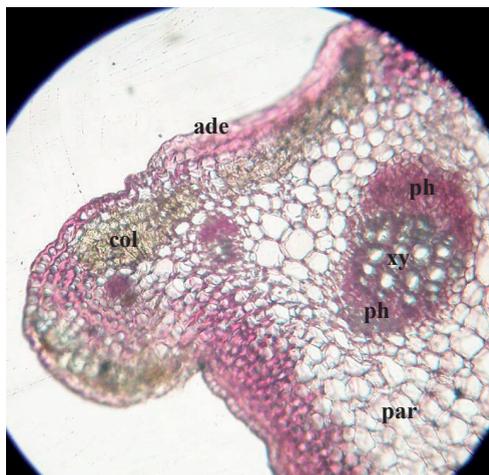


Figure 10. Cross section in the petiole,  
*Iva xanthiifolia*: ade - adaxial epidermis, col - collenchyma, par - parenchyma, xy - xylem, ph - phloem

Non glandular and glandular hairs were not observed in the epidermis of the petiole.

Under the epidermis there are several layers of collenchyma tissue, more developed in the adaxial wings.

Secretory canals are also observed under the collenchyma.

In the fundamental parenchyma there are 3 bicollateral vascular bundles, arranged in a

simple arch shape, one large median and two small lateral ones, surrounded by a parenchymal sheath, with the same structure as those in the stem. Internal phloem is less developed than the external one.

## CONCLUSIONS

On the saline soils from Plopu village, Tulcea county, Romania, the *Iva xanthifolia* grow in compact groups, reaching big heights.

In the structure of the internal cortex of the stem the secretory canals are present.

The vascular bundles in the stem and petiole are of the bicollateral type.

At the level of the internal phloem and at the periphery of the pith, under the vascular bundles, secretory canals were observed.

In both epidermis of the leaf are present glandular and non-glandular hairs, multicellular, uniseriate or biseriate with different sizes.

The leaf is amphistomatic with stomata in both epidermises.

The stomata are of the anomocytic type.

The mesophyll of the lamina is bifacial type with palisade tissue under the adaxial epidermis and spongy tissue under the abaxial epidermis.

In the mesophyll of the lamina, the vascular bundles are of the collateral-closed type.

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## NATIONAL INVENTORY AND PRIORITIZATION OF CROP WILD RELATIVES FROM ROMANIA

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### Abstract

*The paper aimed to present a checklist of wild plant species for use in agriculture, which are native in Romania and contains 525 species. Following an additional selection based on the prioritization criteria (gene traversability, importance and economic use, IUCN status, etc.), the National Priority List containing 275 species was compiled and about 10% of them are preserved in the Suceava Gene Bank. The crop wild relatives represent an important element of the vegetal genetic resources of a nation through their availability for the conservation and sustainable use of their diversity in order to ensure the food security of the country. This national inventory represents the technical support, through which the institutions that manage plant genetic resources strengthen their capacities to implement the national program of conservation of plant genetic resources in accordance with ITPGRFA, FAO, CBD strategies.*

**Key words:** conservation of plant genetic resources, crop wild relatives, checklist, food security, national inventory.

### INTRODUCTION

The Convention on Biological Diversity (CBD), the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA), the Global Plant Conservation Strategy and the Strategic Plan on Biological Diversity, all of which emphasize the need for efficient conservation of plant genetic resources for food and agriculture for counteract the current rate of biodiversity loss at global, regional, national and local levels.

Although farmers have always adapted their cropping systems to unfavorable climatic and environmental conditions, the speed and complexity of the latter indicate another scale of the problems. Thus, a new diversity of cultivated species will be needed to adapt to future extreme environmental conditions.

In this context, farmers need to change their agricultural practices to effectively adapt to climate change, if they are to maintain and improve crop quality and yields. Such practices include adjusting planting times to avoid drought or heat stress and adopting new crop varieties, amongst others (Howden et al., 2007). However, these measures may not be sufficient (Turner and Meyer, 2011), as modern cultivars may lack the ability to adapt to environmental change due to their narrow genetic base, resulting from selection applied in

previous domestication and breeding processes (Stamp and Visser, 2012).

A functional definition of a crop wild relative (CWR) is based on two concepts, the first being the gene pool (Gene Pool) and in the absence of cross-information (gene transferability) and genetic diversity, the second concept is used, that of taxonomic group (Taxon Group).

Thus, Maxted et al. (2006) promoted the following definition: "A crop wild relative of crop plants is a wild plant taxon that has a use derived from its genetic relationship, relatively close to a crop plant; this relationship is defined as belonging to gene groups 1 or 2 or to taxon groups 1 to 4 of the culture species".

Taking into account the genetic variability of these taxa, they can be used as a genetic resource useful in mitigating the effects of climate change on cultivated species, thus helping to maintain and improve productivity and ensure food security (Brozynska et al., 2016).

Factors that affect the entire world biodiversity have a negative impact on the wild relatives of crops and among the most relevant are: genetic erosion, expansion of the anthropogenic area, destruction, degradation, homogenization and fragmentation of natural habitats, use of pesticides and herbicides, changes in practices competition with invasive species and a lack of awareness of the need for conservation and sustainable use of these plant taxa.

Accordingly, it is urgent to take actions to reduce genetic erosion or species extinction.

In situ conservation of CWR in protected areas (Hunter and Heywood, 2011), establishment of genetic reserves (Pinheiro de Carvalho et al., 2012; Fielder et al., 2015a), identification of priorities and efficient collection of samples for ex situ conservation (Khoury et al., 2015; Garcia et al., 2017) are some of the recently approached procedures for CWR conservation. Contextually, compiling the national inventory of CWR is the first essential step in developing the national strategy for the conservation of plant genetic resources, thus ensuring first of all the identification of information needs and the coordination of efforts to conserve and sustainably use these crop wild relatives.

In this regard, a floristic approach was used to generate this national inventory, which initially involved comparing the list of national flora with the list extracted from the PGR Forum Catalog of crop wild relatives for Europe and the Mediterranean (Kell et al., 2007) and selection of those CWRs present in Romania.

To date, as far as we know, seventeen national inventories have been developed by the United Kingdom (Maxted et al., 2007), Portugal (Magos-Brehm et al., 2008), Russia (Smekalova, 2008), Israel (Barazani et al., 2008), Denmark (Bjørn et al., 2011), Spain (Maria Luisa Rubio Teso et al., 2018), Finland (Fitzgerald, 2013), Benin (Idohou et al., 2013), Italy (Panella et al., 2014), Cyprus (Phillips et al., 2014), the Czech Republic (Taylor et al., 2017), the Netherlands (van Treuren et al., 2017), England (Fielder et al., 2015a) and Scotland (Fielder et al., 2015b).

In addition, a global inventory was generated and published (Vincent et al., 2013), two European Catalogs (Heywood and Zohary, 1995; Kell et al., 2005) and a priority checklist of North Africa (Lala et al., 2017).

In Romania, at the moment, there is no database that can be digitally harmonized with the databases of cultivated species, thus, the composition of the national inventory of CWR was done manually.

Romania's flora is estimated at 3795 species and subspecies of higher plants (623 cultivated species and 3136 spontaneous species) (Ciocârlan, 2000), and 37% of plant species are found in meadow habitats, over 700 plant

species are in marine and coastal areas. 4% of plant species are endemic, 75% of them being in the mountain area.

Although, at European level, Romania has the most diversified and valuable natural heritage; the area of protected natural areas of national interest, relative to the area of the country, is 7%. There is no clear evidence of CWR existing in protected areas or outside them.

Regarding the cultivated species, in 2020 the catalog of plant varieties (varieties) that are cultivated on the Romanian territory was made and according to this catalog the number of plant varieties is 2118.

In Romania, are currently cultivated 60 species of plants with human food potential, 22 species with fodder potential, 27 medicinal and aromatic species, 6 species of ornamental plants, 2 species of ornamental shrubs, 25 species of trees and fruit shrubs.

However, high-productivity varieties are known to have a narrow genetic basis and, in many cases, lack the long-term mechanisms of adaptation to extreme environmental conditions (Stamp & Visser, 2012).

These CWRs are often associated with high genetic variability and the idea of food security because they represent the progenitors of today's crop plants, which have in their genetic background, gene sets or even gene complexes that have beneficial traits that confer tolerance to biotic and abiotic factors, which improve nutritional quality and quantity and increase productivity.

Table 1. Some examples of the use of crop wild relative in breeding and the traits they provide. (Mihai D.Cristea, Danela Murariu, 2018)

Crop wild relative	Crop	Target traits
<i>Aegilops triuncialis</i> L.	Wheat	Sources of disease and pest resistance
<i>Avena fatua</i> L.	Wheat	Sources of resistance to drought, disease and
<i>Elymus repens</i> (L.) Gould	Wheat, Barley	Sources of salinity resistance
<i>Sorghum halepense</i> (L.) Pers.	Johnson grass	Sources of disease and pest resistance
<i>Beta trigyna</i> Waldst. & Kit.	Beet	Sources of disease and pest resistance
<i>Linum flavum</i> L.	Flax	Sources of cold resistance
<i>Brassica elongate</i> Ehrh.	Cabbage	Sources of cold resistance
<i>Lathyrus aphaca</i> L.	Grass pea	Sources of disease resistance and productivity

Moreover, CWRs are vital plant genetic resources that, if efficiently conserved and used sustainably, can increase food security, alleviate poverty and improve the stability of natural and semi-natural ecosystems, which have a special role to play in their functioning. and therefore ensures the sustainability of the environment and the continuity of the natural processes within it.

As CWRs have already been used and have been shown to be useful in growing crops (Hajjar & Hodgkin, 2007), increasing knowledge about them and improving their conservation is becoming urgent nowadays.

The purpose of this paper was to develop a CWR checklist of importance in Romania and a priority list of CWR for the implementation of conservation plans and introduction in plant breeding programs to create new varieties and native hybrids that provide increased resistance in relation to climate change, but also to add economic value in response to current consumer market requirements.

To achieve this goal, we have advanced some questions: (i) What criteria should be applied to prioritize CWR in Romania? (ii) What is the degree of national and European threat of CWR from the national priority list?

(iii) Are these species under legal protection in Romania? (iv) What is the degree of endemism of the priority CWR? (v) What is the distribution of their populations and their situation in ex situ conservation in Romania?

This approach has recently been successfully implemented for the implementation of the national inventory of cwr for Spain (María Luisa Rubio Teso, Elena Torres Lamas, Mauricio Parra-Quijano, Lucía de la Rosa, Juan Fajardo, José M. Iriondo, 2017).

## MATERIALS AND METHODS

The whole process of generating the checklist and the priority list of wild relatives of crop plants is presented in Figure 1.

The process involves four stages (two for compiling information and two for setting priorities) thus generating four distinct lists: (1) Basic list of cultivated species genera, (2) List of selected cultivated species, (3) Checklist of cultivated species of crop wild relatives for

Romania (4) Checklist of crop wild relatives of priority for Romania.

### 1. Basic list of genera of cultivated species

According to the PGR Forum project (Kell et al., 2008), in Romania there are over 3000 CWR which represent over 90% of the Romanian Flora, this number being too large to be managed for conservation or sustainable use.

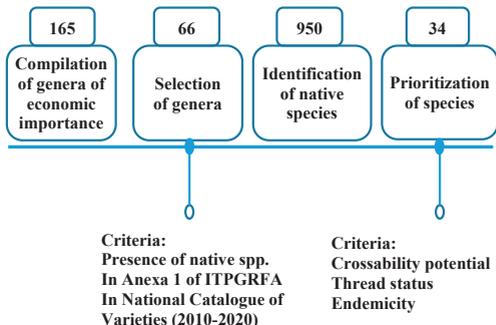


Figure 1. Complete process depicting the steps followed for the generation of the Prioritized Romanian Checklist of Crop Wild Relatives. The process involves four steps (two of compilation of information and two of prioritization) and provides four distinct products: (a) Baseline List of Crop Genera, (b) List of Selected Crop Genera, (c) Romanian Checklist of Crop Wild Relatives and (d) Prioritized Romanian Checklist of Crop Wild Relatives

In this sense, the first step in generating the CWR national checklist was to identify cultivated species that contribute to global food security and are of economic importance, thus obtaining the list of genera that include species cultivated in Romania.

This was done in two stages: (a) compilation of the basic list of cultivated species genera and associated information and (b) selection of cultivated species genera in Romania.

The cultivated species considered were classified into 4 use categories: (1) food, (2) fodder, (3) ornamental and (4) industrial and other uses

The classification of species according to their usefulness was carried out in accordance with Appendix 1. of the International Treaty on Plant Genetic Resources for Food and Agriculture (FAO 2010) and in the Official Catalog of Cultivated Plant Varieties in Romania (of which more information on

production and cultivated area for each species).

To verify the economic importance of other species, compared to those already included, were used as sources of documentation: Union by the International Union for the Protection of New Varieties of Plants (UPOV 2011) and the Germplasm Resources Information Network database of the United States Department of Agriculture (GRIN-USDA 2017) and for ornamental it was used The Community Plant Variety Office (CPVO) (Kwakkenbos pers. comm. 2004).

The UPOV database was also used to collect data on the number of species, infraspecific taxa and / or hybrids associated with a particular crop, specialized publications, plant breeding trends, and other inventories were verified. national CWR.

Thus, the database was compiled with the genera corresponding to these cultivated species and with the information adjacent to them.

## 2. Selection list of cultivated species genera

Following the in-depth analysis of all data, a list of genera was selected based on the following criteria: a) the selected genus must contain at least one species native to Romania; b) the genus must meet at least one of the following conditions: - it contributes to overall food security (found in Annex 1. of the ITPGRFA), - it includes at least one crop species which has at least one variety registered during the 1980s- 2020 in the Official Catalog of cultivated plant varieties in Romania, to highlight the economic importance for the country (Romania).

The resulting list of genera including crop species was evaluated by experts from agricultural institutions who validated all selected genera as well as by consulting databases:

- Global Biodiversity Information Facility, GBIF ([www.gbif.net](http://www.gbif.net));
- The Mansfeld's World Database of Agriculture and Horticultural Crops ([www.mansfeld.ipkgatersleben.de](http://www.mansfeld.ipkgatersleben.de)).

## 3. Romanian checklist of crop of wild relatives

The initial list of CWR in Romania was developed, in the first stage, by selecting geographic criteria (Romania) from the Catalog

of crop wild relatives for Europe and the Mediterranean developed by PGR Forum (Kell et al., 2005).

In order to ensure that all the genera that represent the species cultivated in the country as well as the species studied with them that are found spontaneously on the Romanian territory were included, and were used several documents.

For the identification of plant species from the spontaneous flora of Romania, reference sources were consulted, such as:

- regarding the name of the taxa, it was taken over, in the case of the species from Romanian flora (Ciocârlan, 2000), Atlas of Romanian flora (Mohan and Ardelean, 2011) and Flora RSR, vol. I-XIII, for hybrids and infrataxons;
- Flora Europaea (<http://rbgweb2.rbge.org.uk/FE/fe.html>) and Euro + Med PlantBase (2005) were also consulted to establish the taxonomic rank and synonyms of the taxa (<http://www.euromed.org.uk>);

- the flora lists that can be found in all the management plans of the protected areas (435 sites of community importance, Natura 2000 sites etc);

- articles, studies and monographs of spontaneous and cultivated species.

- articles and books from libraries (public and private), research institutes and botanical gardens were consulted.

The priority-setting process is a first step in any conservation strategy (Maxted and Kell, 2009a,b)

To make this list, the following criteria used proposed by Brehm et al., (2010):

**1. Native status.** This national inventory gives a higher priority to native species in Romania but Non-native species were also included in this list, this decision being based on the importance of these species in the development of the national economy, by increasing the diversity and availability of genetic resources for food and agriculture.

**2. Economic value.** The main use of CWR is to improve the genetic quality of existing crop plants and / or varieties or can be used in the creation of new varieties.

**3. Ethnobotanical value.** Assessment of local knowledge on species uses, thus giving priority to species that are of high importance to local communities.

**4. Global distribution.** The priority is inversely proportional to the distribution, so species that have a low distribution (national or regional) have high priority over species that have a global distribution.

**5. National distribution.** A species that occurs in several areas has been considered rarer compared to a species that occurs throughout the country, so the former will be a priority for active collection and conservation.

**6. In situ and ex-situ conservation status.** If there are species that do not have sufficient genetic diversity conserved in both conservation systems, then these species are a priority for active collection and conservation.

**7. Legislation.** If a species is protected by law then it is a priority for conservation because national governments are responsible for their protection.

**8. Threat assessment.** The status of a species in accordance with the IUCN Red List is probably the most widely used criterion for determining conservation priority. Thus endangered, threatened species are a priority for active conservation and collection.

The following documentation materials were used in compiling the Romanian checklist of crop of wild relatives and for applying the selection criteria:

- IUCN threat category ([www.iucnredlist.org](http://www.iucnredlist.org)).
- number of infraspecific taxa belonging to species included in the Red List of Endangered Species in Romania (Oltean et al., 1994), Red Book of Vascular Plants in Romania (Dihoru and Negrean, 2009), Carpathian List of Endangered Species (Krzysztof Kukuła et al., 2003), Carpathian red list of forest habitats and species carpathian list of invasive alien species (Ján Kadlečík, 2014),
- cross-breeding potential (gene transmission) was evaluated according to the concept of genofond issued to Harlan and de Wet (1971) (possibility of gene transfer between wild and cultivated species) and the concept of the taxon group by Maxted et al., (2006) (assimilation of the taxonomic hierarchy to the concept of the genetic basis).

For the identification of native species in Romania were used:

- Flora RSR, vol. I-XIII. (<https://www.cwrdiversity.org/checklist/>),

-the Germplasm Resources Information Network database of the United States Department of Agriculture (<https://npgsweb.arsgrin.gov/gringlobal/taxon/taxonomysearchcwr>) or additional references.

- The Cwr Catalog For Europe And The Mediterranean (Kell, 2005).

For the compilation of cross-breeding potential and genetic background data, a complementary list of cultivated species was generated for each selected genus using as a reference the database of the US Department of Agriculture's Germoplasmic Resource Information Network (GRIN-USDA, 2017).

Same database (<https://npgsweb.ars-grin.gov/gringlobal/taxon/taxonomysearchcwr>) was used to determine the degree of kinship (at the level of the gene pool and / or the taxonomic group) between the CWR and the cultivated species.

#### **4. List of crop wild relatives priority for Romania**

Regarding the compilation of the priority list of the CWR, there is some consensus in combining three criteria: a) the potential for economic capitalization of the related crop species, b) the potential for crossing (degree of kinship of the CWR with the crop species / ease of passage CWR in culture, in relation to the genofond and / or group of taxa), c) the relative level of threat and endemism (for food, feed, industrial and ornamental plants).

However, regardless of the priority setting methodology and criteria used, the total number of priority CWRs should be adjusted to a number that can be actively conserved using available financial and human resources. There is no precise way to estimate the number of priority CWRs because the estimate would be subjective.

A more flexible approach would be to assign different levels of priority for conservation, depending on the priorities of the institutions that will carry out the CWR conservation and the use of a reasonable number of taxa for each of these institutions, to implement active conservation.

Continuing to use this approach, some taxa, which are not yet an immediate priority for conservation, may appear in the same sites as

those with high priority, so they may be included in the same

In view of the above, it has been established that the priority CWR species are those found in the primary gene pool 1 (in which gene transfer is free, where the cultivated and wild forms of the crop plant are found) and those in secondary gene pool 2 (gene transfer is possible using conventional breeding techniques) (Harlan and de Wet, 1971) or CWR species belonging to group of taxa category 2 (same series or section as crop plant) and 3 (same subgenus as a crop plant) (Maxted et al., 2006). In prioritizing CWR, the concept of gene pool has always been a priority over the concept of the taxon group, but when information on cross-species was not available, the taxon concept was applied.

Another specific element in compiling the CWR priority list was the selection of all species for direct food use, forage potential species, industrial species, which are found in any of the IUCN threat categories (critical, endangered species, vulnerable and almost threatened) or endemic to Romania.

Thus, the resulting list was verified in relation to: Red List of higher plants in Romania, Directive no. Council Directive 92/43 / EEC of 21 May 1992 on the conservation of natural habitats and of wild flora and fauna (Annexes II and IV), European Red List of Medicinal Plants, European Red List of Vascular Plants and Convention on the conservation of European wildlife and natural habitats (Bern, 1979).

The inclusion of species in this national inventory provides these species with legal protection that involves the design and implementation of appropriate conservation plans as well as the constant assessment and monitoring of their conservation status.

Finally, all the priority species were verified, worldwide, in the databases of plant genetic resources: - the database of the Bank of Plant Genetic Resources "Mihai Cristea" Suceava; - the EURISCO catalog (EURISCO 2020); - the Germplasm Resources Information Network database of the United States Department of Agriculture (GRIN-USDA, 2020); - the GENESYS Global Portal on Plant Genetic Resources (GENESYS, 2017).

The development and improvement of the national "ex situ" collection of the CWR requires an urgent collection, the priority species in this respect are those found in gene pool 1b and 2 or taxon group 2, which is under any threat category according to the International Union for Nature Conservation (IUCN), species that have the status of endemic to Romania as well as those species that have less than five entries (germplasm samples) in the Plant Genetic Resources Bank "Mihai Cristea" Suceava. This threshold of five entries is considered to be the minimum number of populations needed to conserve intraspecific genetic diversity (Brown and Briggs, 1991). The priority for CWR collection provides the following criteria:

- (1) Urgent priority. They are CWR species present in the primary or secondary genetic background as well as in the group of taxa of the crop species, are endemic or threatened and have no representation in gene banks;
- (2) Urgent. The species is not represented in gene banks;
- (3) To be collected. The species has less than five populations represented in Gene Bank;
- (4) Not a priority for collection. The species has over five Gene Bank entries for each species.

## RESULTS AND DISCUSSIONS

The basic list of genera associated with cultivated species comprises 165 genera.

The genus selection list associated with cultivated species used in the generation of the CWR Checklist contains 66 genera.

The distribution of the genera in this list according to the category of use of the species is as follows:

- food species include 41 genera included in 12 families (*Brassicaceae*, *Fabaceae* - 26 species, *Liliaceae* - 27 species, *Poaceae* - 20 species, *Rosaceae* - 30 species);
- fodder species include 20 genera included in 2 families (*Poaceae* - 54 species, *Fabaceae* - 71 species);
- ornamental species include 1 genus included in a family (*Rosaceae* - 16 species);
- industrial and other uses include 6 genera included in 4 families (*Lamiaceae* - 18 species).

The families Fabaceae and Poaceae are the most important, having 15 and 17 genera respectively, followed by *Brassicaceae* with 8 genera.

The checklist of wild relatives of crop plants comprises a total of 940 species. The category of food species includes 356 species, the category of feed species has 350, the ornamental ones 97 species, and the industrial and those with other uses are 131 (Figure 2).

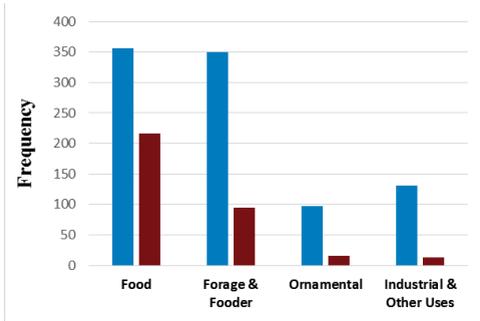


Figure 2. Number of species in the Checklist of wild relatives of crop plants and Priority list of wild relatives of crop plants, ordered by categories of use

This checklist of species together with information on priority use, taxonomic classification, concept of genofond group of taxa, threat status, endemism and number of chromosomes are to be integrated into a database, which will later be available online. Because the gene pool information was found for 128 of the 350 species, the others were assigned the group of taxa corresponding to the category.

### Romania's priority list of wild relatives of cultivated plants

Applying the agreed criteria to each use category has reduced the number of species from the list to 328 (34% of the original checklist).

The Romanian priority list of wild relatives of crop plants contains 162 species related to crop species for food use, 124 species with forage value, 15 ornamental species and 23 industrial species and other uses (Figure 2).

All priority species, together with all the information gathered during the selection process will be available online.

According to the prioritization criteria used, the selected species belonged mainly to the primary (60 species) or secondary genes (42

species), but also to the group of taxa (28 species to the primary group of taxa and 39 species to secondary group of taxa - the same section or subsection as the crop species) (Figure 3a).

Three of the priority species are endemic in Romania. Over 8% of the prioritized species (27 species out of 328) are classified in one of the IUCN threat categories at the national level (including the almost endangered category Fig. 3b), and 15 species are threatened in the Carpathian area (Table 2).

In addition, 65 species from the Romanian Priority List of wild relatives of cultivated plants are included in the Red List of higher plants in Romania (Oltean, Negrean et al. 1994), The Red Book of vascular plants in Romania (Dihoru, Negrean, 2009 ).

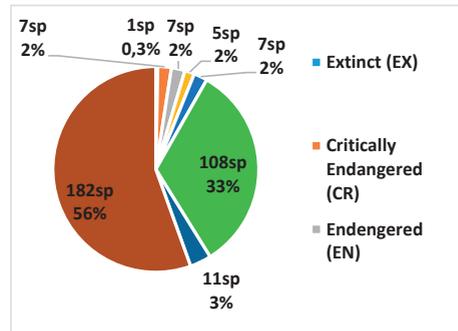
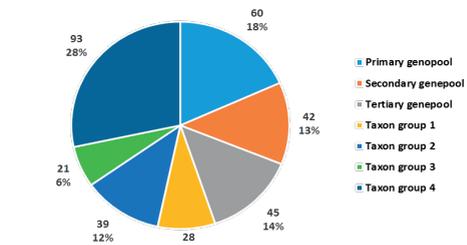


Figure. 3: a) Classification of priority species according to the genetic background or group of taxa of Harlan and Wet (1971) and Maxted and colab. (2006); b) The number of species in any of the threat categories according to Red list of superior plants from Romania: Oltean, M. (coord.), According to the Union Criteria International Organization for Conservation of Nature (IUCN

Regarding the *ex situ* conservation, Suceava Gene Bank has 105 species in the asic collection (+ 4 °), approximately 32% of the

priority CWR, so 219 species are not represented in the national collection of plant genetic resources.

Analyzing this situation, less than 89% of the species represented in the national collection of plant genetic resources managed by the Plant Genetic Resources Bank "Mihai Cristea" Suceava have 20 or more entries, while 69% of these species have between 1 and 4 entries.

A total of 58 species are of primary importance, because they are endemic to Romania, they are threatened according to the IUCN, they belong to the primary or secondary genofond or group 2 of taxa, as well as represented by less than five entries in the BRGV Suceava collections.

Of these, twenty-eight have no representation in BRGV Suceava (Priority 1).

One hundred and eight (108) species are found in priority collection category 2 (Emergency. Species are not represented in gene banks), 21 in priority collection category 3 (Need for collection. Less than five populations represented in gene bank) and finally 141 in priority collection category 4 (no priority for collection) All this information will be available for each species in the Romanian priority list of the CWR.

Romania's national strategy for CWR conservation aims at the long-term active conservation of CWR taxonomic and genetic diversity but, at the same time, promotes the use of these resources, because experience has shown that sustainable conservation is achieved through use.

In particular, with regard to the *in situ* conservation of CWR, once conservation sites have been established (genetic reserves and informal *in situ* conservation sites), they can be grouped into a coherent national network, thus providing the opportunity to monitor and evaluate short- and long-term changes in CWR diversity.

This national CWR conservation strategy is a major objective of the CBD 2020 Strategic Plan, COP 10 decision X / 2 (Nagoya, Japan, October 2010).

Development of the Romanian Checklist a wild relatives of crop plants was made following the "list of crop species", although many countries use the national floristic list.

This method was approached because the European Catalog of the CWR indicates about 3,000 CWR are present in Romania (Kell et al., 2008), which represents more than 80% of flora in the country.

Many species in this catalog are already administered by other public administration interest groups, e.g. forest species, which have their own national inventory and conservation program, as well as medicinal and aromatic plants. With this in mind, the CWR checklist was generated directly from a list of crop species that are important for the efficient use of economic resources and to avoid duplicates and overlaps with species that are already managed by the public administration.

In order to simplify the procedure for identifying CWR, a list of crop species has been compiled in accordance with national and regional socio-economic criteria. Thus, we focused on the most important CWR for Romania and, at the same time, without neglecting the crop species that contribute to the country's economy and food security worldwide. This approach was also followed by Berlingeri and Crespo (2012) in Venezuela, Idohou et al. (2013) in Benin, Maria Luisa Rubio Teso et al. (2017) in Spain, it can be a valid alternative to extract the most important CWRs from a wide list of species present in a nation. Non-native species were excluded from the checklist of wild relatives of cultivated species in Romania because a large number of CWRs are naturally present in our country, requiring the establishment of strict criteria for the prioritization of these species.

On the other hand, in this list of CWR, species that are far from their centers of diversity - centers of origin (geographical areas where the species has a higher degree of variation and where there is significant genetic variability) have been introduced. represented by alleles " (Corinth, 2014)] because these species lack high genetic variability, which is of fundamental importance in reproduction.

However, non-native species with a source of genetic variation have been included in the Romanian CWR checklist, noting that these taxa have only been introduced at the species level.

Table 2. CWR species on the Romanian Prioritized Checklist (Food and Forage & Fodder categories) threatened at the Carpathian level and their corresponding status at the national level

Species	Family	Red List Carpathian Status	Red List Romanian Status
<i>Castanea sativa</i> Mill.	Fagaceae	EN	NT
<i>Astragalus pseudopurpureus</i> Gusul.	Leguminosae	EN	EN
<i>Trifolium lupinaster</i> L.	Leguminosae	CR	EN
<i>Astragalus roemeri</i> Simonk.	Leguminosae	EN	NE
<i>Lactuca aurea</i> (Vis. & Pančić) Stebbins	Asteraceae	EN	NE
<i>Barbarea vulgaris</i> W. T. Aiton	Brassicaceae	CR	DD
<i>Phleum subulatum</i> (Savi) Aschers. & Graebn.	Poaceae	EN	DD
<i>Ribes spicatum</i> E. Robson	Grossulariaceae	CR	NE
<i>Festuca filiformis</i> Pourr.	Poaceae	EN	DD
<i>Barbarea stricta</i> Andr. ex Besser	Brassicaceae	CR	DD
<i>Pisum elatius</i> L.	Leguminosae	CR	DD
<i>Astragalus depressus</i> L.	Leguminosae	CR	DD
<i>Agropyron cristatum</i> subsp. <i>sabulosum</i> (L.) Gaertn.	Poaceae	VU	VU
<i>Aegilops triuncialis</i> L.	Poaceae		
<i>Armoracia macrocarpa</i> Baumg.	Brassicaceae	VU	DD
<i>Carthamus lanatus</i> L.	Asteraceae	EN	DD
<i>Allium obliquum</i> L.	Alliaceae	EN	DD
		CR	NE

Red List of Superior Plants in Romania (Oltean, Negrean et al., 1994), Red Book of Vascular Plants in Romania (Dihoru, Negrean, 2009), Carpathian List of Endangered Species (Krzysztof Kukula et al. 2003), Carpathian red list of forest habitats and species carpathian list of invasive alien species (Ján Kadlecík, 2014).

Consequently, the CWR checklist, with reference to Romania (940 species) is higher than the list generated by Spain (926 species), but lower than those generated for other countries, such as Finland (1905 taxa), the Sea Great Britain (1955 species), Portugal (2261 taxa), USA (2495 taxa), Czech Republic (3283 species) or China (almost 24,500 species).

Generating an initial list identifying CWRs also involves further prioritizing them in order to take direct action on the conservation and sustainable use of these plant genetic resources. These data will be available on the web, which will allow you to make potential taxonomic updates / changes and changes in the evaluation of the list of the most important wild relatives of crop plants in Romania.

This lack of available information on direct crossover experiments is consistent with the results found in previous studies (Kell et al., 2014; Fielder et al., 2015a, 2015b).

Although the concept of the taxon group can be a useful decision-making proxy when genepool information is not available, these results clearly show that cross-breeding experiments between cultivated species and their wild relatives are essential to assess and facilitate the potential use of CWR, namely in the introgression of genes useful in the genome of cultivated species.

The criteria for prioritizing the CWR in Romania were the same as those used by other countries, namely those related to the concept of crossability, degree of threat and endemism.

In the CWR priority list, only 17% of all identified species have high crossability, these species are present in the primary gene pool of crop forms, data normally provided by plant breeders. However, these data are often considered confidential information and are not available as published material.

Where information on direct cross-breeding experiments between CWR and crop plants, which are essential for the evaluation and potential use of CWR in plant breeding, is not available, data on the taxonomic group of crop species in relation to CWR may be used.

Evaluating the degree of threat of CWR from the priority list, we find that approximative 20% of them fall into a category of threats described in the IUCN Red List.

This percentage is higher compared to other countries such as Cyprus (9%), Germany (16%), Lithuania (16%), Norway (13%), Great Britain (12%) and even compared to the large flora of China (17%), but lower compared to countries such as the Czech Republic (54%), Finland (71%), Jordan (32%), Portugal (65%) or Spain (23%).

However, endangered species at European level that have not been assessed in the Romanian Red List should be assessed in future editions of the Romanian Red List of Vascular Flora.

The publication of this list can also be very useful in subsequent reviews of the National Catalog of Threatened Species to include all priority CWRs that are endangered and ensure their legal protection.

The inclusion of CWR in the Romanian Strategy on the Conservation of Plant Genetic Resources will be an important step in recognizing the importance of this category of plants, at national level, even if the implementation of active conservation plans is the responsibility of local communities.

For the genetic diversity of a species to be well conserved, that species should have a germplasm in a Gene Bank of at least five entries from five different populations (according to Brown and Briggs (1991) and Maxted et al., (2008).

However, recent studies suggest that this criterion of at least five populations per species should be replaced, in the long run, with a more ambitious goal in which the number of entries to be collected is estimated on the basis of species, in proportion to genetic diversity of that species. Based on this premise, in addition to prioritizing the collection of the 168 species on the priority list that do not have accessions in Gene Bank, CWR collection should also focus on improving the representation of species that are already conserved to obtain the minimum population sampled to represent their genetic diversity.

Of these, 58 species on the priority list that are endemic, threatened and have less than five gene bank accessions should have the highest priority.

Given the above criteria, Whitlock et al., (2016) propose that over 35% of populations be kept in line with the recommendations of the Convention on Biological Diversity (CBD).

Although this study aims to implement active *in situ* conservation plans, it could also be applied in *ex situ* conservation, as its major objective is to preserve sufficient genetic diversity to adequately represent the species. The safest method of preservation is the *ex situ*, in Gene Bank, where the germplasm is stored in optimal conditions of temperature and humidity. The optimal genetic diversity of a species stored in

Gene Bank is different from species to species, taking into account the type of multiplication of the species and the distribution and size of populations along with their environmental conditions (Brown & Marshall, 1995).

In addition, the use of existing molecular data can also help in correctly determining the minimum number of accessions that may represent genetic diversity within a species (Camadro, 2012).

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## CONCLUSIONS

Romania's biodiversity, which also includes existing CWR species, must be conserved using strategies and action plans based on the establishment and management of protected areas and sustainable human use of the rest of the territory, as well as species-specific approaches.

Therefore, the correct identification and setting of the priority CWR is essential. This List of wild relatives of crop plants in Romania must be managed coherently and coordinated by the agriculture and environment departments of the public administration and continuously reviewed in a participatory way to include species with real potential to meet the needs and ever-changing trends in agriculture and plant growth.

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## EVALUATION OF BREEDING INDICES FOR DROUGHT TOLERANCE IN ALFALFA (*Medicago*) GENOTYPES

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### Abstract

*The goal of the study is to find out the best indices for drought tolerance evaluation in alfalfa genotypes to distinguish the best ones for further use in the plant breeding process for drought resistance. The study was carried out during 2017-2020 at the experimental field of the Institute of Irrigated Agriculture of NAAS (Kherson, Ukraine) with accordance to modern standards of scientific work in agronomy. We studied 24 varieties and populations of alfalfa in the conditions of optimal (irrigation) and stress (rainfed) humidification in the South of Ukraine, which is characterized as a semi-arid climatic zone. To evaluate the resistance of the studied genotypes of the crop to drought, 14 different indices were applied. Based on the results of the study, new index for drought tolerance evaluation in alfalfa, named stress resistance index (ISR), was introduced. We selected five genotypes: M.g./P.p., LR/H, Ram.d., M.g./CP-11 and M.agr./C., which had the highest yield of 8.30-8.47 kg/m<sup>2</sup> under the moisture stress as the most prospective for their further use in the plant breeding process. Four indices, namely, yield index (YI), mean geometric productivity (GMP), harmonic mean productivity (HMP), stress sensitivity index (SSI) and the developed stress resistance index (ISR) were selected as the best ones for characterization of alfalfa varieties by drought resistance as they do not only characterize the genotype in terms of its drought resistance, but also in terms of productivity under the stress conditions.*

**Key words:** alfalfa, drought resistance indices, productivity, stress conditions, yield.

### INTRODUCTION

Alfalfa is a perennial forage crop, which is cultivated all over the world, and it is characterized, in comparison to other forage legumes, by high biomass productivity, nutritional value with high protein content. Alfalfa helps to increase soil fertility (Latrach et al., 2014), protects soils from wind and water erosion (Abdelguerfi & Abdelguerfi-Laouar, 2002), and increases the resilience of crop production and livestock systems (Annicchiarico et al., 2011). Besides, the fixation of atmospheric nitrogen makes it the best forage crop for other crops.

Alfalfa grows in a wide range of climatic conditions, from the equator to almost the Arctic polar circles (Annicchiarico et al., 2011). According to numerous forecasts, global climate change will lead to higher temperatures, changes in the geographical structure of precipitation and in the future to an increase in the frequency of extreme climatic events (Aleksandrov, 2002; Harrison et al., 2014), which is already observed in southern

Ukraine. Abiotic stresses are the main factors that reduce crop productivity. Drought is the most significant, as it limits the capabilities of agricultural plants, reducing their productivity in arid and semi-arid areas (Hussain et al., 2012; Mollasadeghi et al., 2011). The intensity and severity of the drought can affect a sensitive and strategic sector, such as agriculture, which can threaten food security. The detrimental effects of abiotic stress are a serious limitation for cultivation of this crop (Vasconcelos et al., 2008; Wang et al., 2015). But due to its strong and branched root system, it is considered to be a crop with high drought resistance and wide adaptability to arid conditions (Lemaire, 2006; Li et al., 2020). However, like any other crop, it reacts negatively to drought and, in order to adapt and survive under stress, it undergoes morphological, physiological, biochemical or molecular changes, which must be taken into account when creating drought-resistant varieties while increasing yields and product quality. During the dry season, alfalfa plants (*Medicago*) reduce the aboveground vegetative

mass (Bellague et al., 2016; Durand, 2007), which limits the leaf area index, and consequently reduces the productivity of biomass. Therefore, to stabilize and increase the productivity of alfalfa, it is necessary to increase the drought resistance of alfalfa plants, and the study of this trait is an important step in plant breeding programs (Yu, 2017). The amount of moisture loss through the evapotranspiration is steadily increasing, and this trend will only worsen in the future (Aleksandrov, 2002), so declining yields are a major problem and at the same time a basis for plant breeders to strengthen crops to adapt to climate change and increase their productivity under the stressful conditions (Cattivelli et al., 2008). Identification and development of drought-resistant genotypes is one of the main tasks of plant breeding programs, but the creation of high-yielding varieties and the realization of their yield potential in arid conditions is an extremely difficult task for plant breeders (Mustatea et al., 2003; Richards, 2006; Richards et al., 2002). The development of drought-resistant varieties is hindered by low heredity of traits and lack of effective selection strategies (Kirigwi et al., 2004). The selection of drought-resistant populations is difficult due to the strong interaction between genotypes and the environment and the limited knowledge of the functions and role of resistance mechanisms. Different researchers have used different methods to assess genetic differences in drought resistance. Some researchers believe that it is reasonable to conduct the selection of genotypes only in favorable conditions (Betran et al., 2003), and others – in arid conditions (Ceccarelli, 1987; Ceccarelli & Grando, 1991). However, there are many researchers who have chosen both paths and use the selection of genotypes in both favorable and stressful conditions (Clarke et al., 1992; Fernandez, 1992). Plant susceptibility to drought is defined as a function of reduced yields under water stress (Koleva & Dimitrova, 2018), compared to potential yields (Ramirez & Kelly, 1998). Therefore, different breeding indices are used to differentiate genotypes from drought resistance, which are based on plant productivity in optimal and stressful conditions (Fisher & Maurer, 1978; Lin & Binns, 1988), and are used to select drought-resistant

genotypes (Boussen et al., 2010; Mitra, 2001; Zou et al., 2007).

Rosielle et al. (1981) proposed to use the Tolerance Index (TOL) as the difference between yield under irrigation and yield under natural humidification, and the mean productivity (MP), as the arithmetic mean of yield under stress and optimal conditions. Blum (1988; 2005) determined the Drought Resistance Index (DI), which was generally accepted to determine the genotypes that provide high yields, both under stress and in better conditions. Fisher & Maurer (1978) recommend the use of a Stress Sensitivity index (SSI) to determine the stability of plant productivity, which records the value of yield in optimal and stressful conditions. The Stress Sensitivity Index (SSI) is a good one for identifying high-yielding genotypes that are also highly resistant to stress. As a rule, a lower level of SSI indicates less variation in crop yield under stress and under optimal conditions. Fernandez (1992) recommends using the Stress Tolerance Index (STI), and Saba et al. (2001) recommended its use in plant breeding programs for screening high-yielding genotypes in conditions of stress and its absence. Stable varieties have higher values of this index. Studying the yield of genotypes of mung beans (*Vigna radiata* L.) in stressful and optimal environments, Fernandez (1992) classified them into four groups:

- group A - varieties that have equally high productivity in both environments;
- group B - varieties that have high productivity only in optimal conditions;
- group C - varieties with high yields under stress;
- group D - varieties with low yields in both environments.

To determine the sensitivity of varieties to stress due to different drought intensities in different years Fernandez (1992) and Schneider et al. (1997) proposed the use of Geometric Mean Productivity (GMP) of the varieties in both environments. Besides, Gavuzzi et al. (1997), Bousslama & Schapaugh (1984) and Choukan et al. (2006) proposed to use the Yield Index (YI), the Yield Stability Index (YSI) and the Yield Reduction Index (YRI), respectively. During the study of the drought resistance indices of corn, Moghaddam & Hadizadeh

(2002) stated that a low Tolerance Index (TOL) does not necessarily mean a high yield of a variety under stressful conditions, because the yield of a certain variety may be low under optimal conditions and show less reduction under stress, which leads to a decrease in TOL and this variety can be defined as drought resistant. However, Fernandez (1992) believed that the TOL and SSI indices reflect the drought resistance of the variety better. The use of the SSI index to identify drought-resistant varieties is a false direction. They believe that because the formula for the index calculation used the share of yield of a particular variety under stress to optimal conditions, as well as the ratio of productivity in stress to non-stress conditions in all varieties, the two varieties with high or low yields in both environments may have the same SSI value. With regard to MPI, the authors found that the use of a Mean Productivity Index often leads to the selection of varieties with high yields under optimal conditions that are less tolerant to stress. Malek-Shahi et al. (2009) presented MPI as an appropriate index for determining drought-resistant varieties. Shirani Rad & Abbasian (2011) while studying the sensitivity to stress in six varieties of winter oilseed rape found out that the indices of GMP, STI and MPI are the most appropriate indices for the determination of drought-resistant varieties. The same opinion is held by Sio-Se-Mardeh et al. (2006), which point out the importance of GMP, STI and MPI indices as the most effective ones for the exclusion of the varieties with high yields in both dry and optimal conditions (Yarnia et al., 2011).

In order to increase the effectiveness of the STI index, Farshadfar & Sutka (2002) proposed modified stress resistance indices (M1STI, M2STI), which adjust the STI. For screening drought-resistant genotypes in different environmental conditions, Moosavi et al. (2008) developed the percentage of sensitivity to stress index (SSPI).

Hao et al. (2011) recommend the index as an integrated selection criterion (SI) because it provides an assessment of drought stress resistance based on yield and related agronomic characteristics and will thus be useful for determining drought-resistant genotypes in plant breeding programs (Khalili et al., 2016).

Therefore, there are 14 indices for determining the drought resistance of genotypes, which we used in our studies. The goal of the study is to evaluate the response of varieties and populations of alfalfa in different environments and determine the best one not only by drought resistance but also by productivity under stress for their further use in the plant breeding process, and to select indices that allow distinguishing the genotypes with such traits.

## MATERIALS AND METHODS

The study was conducted at the Institute of Irrigated Agriculture of NAAS (Ukraine, Kherson, vil. Naddnipyrianske, 46°44'50.1"N 32°42'30.0"E), located on the Ingulets irrigated array, in 2017-2020 in the field conditions. The object of the study were varieties and populations of alfalfa: Unitro, Elehiya, Prymorka, M.g./P.p., Sin(s)/Prymorka, LR/H, Prymorka/Sin(s), A.-N. d. № 114, A.-N. d. № 15, A.-N. d. № 38, D. k.s. Ram. d., (Emeraude/T.)2, T./Emeraude, M.g. CP-11, M.agr./C., A.r. d., M.g./M.agr., M.g. d., FHNV<sup>2</sup>, B.11/P.d., Zh./TsP-11 at forage use under two conditions of humidification: irrigation (drip irrigation) and natural humidification on the herbage of the first and second years of use. Productivity and drought resistance were determined using different indices:

$$MPI = \frac{Y_p + Y_s}{2} \quad \text{Rosielle \& Hamblin, (1981)} \quad (1)$$

Where: MPI - mean productivity index,  $Y_p$  - yields at the optimal conditions,  $Y_s$  - yields at the stress conditions.

$$SSI = \frac{1 - \frac{Y_s}{\bar{Y}_p}}{1 - \frac{\bar{Y}_s}{\bar{Y}_p}} \quad \text{Fisher \& Maurer, (1978)} \quad (2)$$

Where: SSI - stress sensitivity index,  $\bar{Y}_p$  - mean productivity of all the varieties in the optimal conditions,  $\bar{Y}_s$  - mean productivity of all the varieties in the stress conditions.

$$TOL = Y_p - Y_s \quad \text{Rosielle \& Hamblin (1981)} \quad (3)$$

Where: TOL - index of drought toleramce.

$$YSI = \frac{Y_s}{\bar{Y}_p} \quad \text{Bousslama \& Schapaugh (1984)} \quad (4)$$

Where: YSI - yield stability index.

$$YI = 100 \times \frac{Y_s}{\bar{Y}_s} \quad \text{Gavuzzi et al. (1997); Lin et al. (1986)} \quad (5)$$

Where: YI - yield index.

$$STI = \frac{Y_s \times \bar{Y}_p}{\bar{Y}_p^2} \quad \text{Fernandez (1992)} \quad (6)$$

Where: STI - stress tolerance index.

$$GMP = \sqrt{Y_s \times \bar{Y}_p} \quad \text{Fernandez (1992); Schneider et al. (1997)} \quad (7)$$

Where: GMP - mean geometric (proportion) productivity.

$$RDI = \frac{\frac{Y_s}{\bar{Y}_p}}{\frac{\bar{Y}_s}{\bar{Y}_p}} \quad \text{Fischer \& Maurer (1978)} \quad (8)$$

Where: RDI - relative drought tolerance index.

$$DI = \frac{Y_s \times \left(\frac{Y_s}{\bar{Y}_p}\right)}{\bar{Y}_s} \quad \text{Lan (1998)} \quad (9)$$

Where: DI - drought tolerance index.

$$SSPI = 100 \times \frac{Y_p - Y_s}{2 \times \bar{Y}_p} \quad \text{Moosavi et al. (2007)} \quad (10)$$

Where: SSPI - stress sensitivity predisposition index.

$$M_1STI = STI \times \left(\frac{\bar{Y}_p}{\bar{Y}_p}\right)^2 \quad \text{Farshadfar \& Sutka (2002)} \quad (11)$$

$$M_2STI = STI \times \left(\frac{Y_s}{\bar{Y}_s}\right)^2 \quad (12)$$

Where: M<sub>1</sub>STI, M<sub>2</sub>STI - modified indices of the stress tolerance.

$$ATI = \frac{Y_p - Y_s}{\bar{Y}_p} \times \sqrt{\bar{Y}_p \times Y_s} \quad \text{Moosavi et al. (2007)} \quad (13)$$

Where: ATI - abiotic tolerance index.

$$HMP = 2 \times \frac{Y_p \times Y_s}{Y_p + Y_s} \quad \text{Kristin et al. (1997); Chakherchaman et al. (2009); Jafari et al. (2009)} \quad (14)$$

Where: HMP - harmonic mean productivity.

Statistical processing of the experimental data was performed using AgroSTAT, XLSTAT, Statistica (v. 13) software at p<0.05.

## RESULTS AND DISCUSSIONS

Weather conditions over the years of the study differed both in temperature and in the amount and characteristics of precipitation, which made it possible to analyze varieties and populations of alfalfa for resistance to stress (drought) conditions. The hydrothermal coefficient (HTC) in 2017, 2018 and 2020 fluctuated between 0.51-0.55, which indicates very arid climatic conditions, while in 2019 it was 0.88, which belongs to arid conditions. Analysis of resistance of the alfalfa varieties and populations to stress was performed using 14 different drought tolerance indices: MPI, SSI, TOL, YSI, YI, STI, GMP, RDI, DI, SSPI, M<sub>1</sub>STI, M<sub>2</sub>STI, ATI, HMR and ISR stress resistance index, which was developed by our scientific group.

In the plant breeding for drought resistance, an important aspect is not only the resistance of plants to drought, i.e., the ability of plants to tolerate significant dehydration and overheating and survive drought with the lowest yield reduction, but also to show the maximum productivity under the stress. For example, genotypes may show a slight decrease, i.e., the difference in the yield obtained under optimal and stress conditions, but also low productivity under the stress. On the contrary, the population has high productivity during droughts, but a greater difference between yields in the optimal and stress conditions. For convenience, alfalfa populations are divided into three groups according to yielding capacities. Under irrigation (Y<sub>p</sub>) the populations with yields above 20.00 kg/m<sup>2</sup> were classified as high, 19.00-20.00 with medium and below 19.00 kg/m<sup>2</sup> - with low yields, under the stress conditions (Y<sub>s</sub>) - with a yield of more than 8.00 kg/m<sup>2</sup> - with a high,

7.00-8.00 - medium and below 7.00 kg/m<sup>2</sup> to the group with a low yield.

The obtained experimental data allowed us to identify 5 populations of alfalfa by the yields in the stress conditions: M.g./P.p., LR/H, Ram.d., M. g./CP-11 and M.agr./C. with a herbage yield of 8.30-8.47 kg/m<sup>2</sup> with a high MPI (13.82-14.37), which shows the potential yield of the genotype in different cultivation conditions. In the variety Elehiya and population B11/P.d. there is also observed a high MPI (14.07; 13.94), which was formed because of high yields under the optimal conditions (irrigation), but low or medium - under the stress, therefore, the MPI cannot be considered as a reliable index for the determination of the resistance of genotypes to stress.

The drought sensitivity index (SSI) ranged from 0.91 to 1.12. It characterizes how sensitive the genotype is to the effects of drought and the lower the rate is, the greater the drought resistance of the genotype is. Nine populations were identified according to this index: M.g./P.p., LR/H, Ram.d., M.g./CP-11 and M.agr./C., Prymorka/Sin(s), Zymostiyka/MK, A.r. d., M.g./M.agr. with its value of 0.91-0.96. But the last four genotypes entered this group due to low yields under the optimal conditions and mean ones under the stress. Therefore, we believe that this index can be used to determine drought resistance of genotypes, but in combination with other indices, to identify stress-resistance and high drought productivity more accurately.

The Drought Tolerance Index (TOL) and the Stress Sensitivity Predisposition Index (SSPI) are similar in nature and show the loss of yield under the influence of drought, the first in absolute units, the second in percent.

The population M.g./M.agr. is characterized with the lowest TOL - 10.35 and predisposition to the stress SSPI - 26.61. Its low yield of 17.95 kg/m<sup>2</sup> in the optimal conditions and a mean of 7.60 kg/m<sup>2</sup> under the stress does not mean that it is more resistant to drought and has greater productivity under the stress than the population M.g./P.p. and M.g./CP-11 with TOL indices 11.52 and 12.14, and SSPI 29.62 and 31.22, which according to the indices do not belong to drought-resistant but formed a high yield under the stress - 8.44 and 8.33 kg/m<sup>2</sup>. Therefore, low TOL and SSPI will mean stress

resistance, but there is a very high probability that the population will be more productive under the stress, although with the best TOL and SSPI indices will not be allocated as drought resistant.

According to the yield stability index (YSI), i.e., the ratio of the yield under the stress to the yield under the optimal conditions, with fluctuations from 0.31 to 0.44, ten populations were significantly distinguished: FHNV<sup>2</sup>, M.g./CP-11, A.p.d., M.g./M.agr., M.agr./C., Zymostiyka/M.K., Ram.d., Prymorka/Sin(s), M.g./P.p., LR/H, in which this index varied within 0.40-0.44, but, as in the previous index, the mean yield under the stress and low under the optimal conditions led to high index rates in some populations. This means that the YSI index should be used only in comparison with others, because more productive populations, both under the optimal conditions and drought, may not be included into the group of drought resistant.

Yield index (YI), geometric mean productivity (GMP) and harmonic mean productivity (HMP) express the yield of a particular genotype under the stress conditions to the mean yield of the studied genotypes in these conditions, but the indices YI, GMP, HMP are calculated using different formulas. It is believed that they are less sensitive to large differences between the values of potential yields and yields under stressful conditions.

According to these indices, there were five populations distinguished: M.g./P.p., LR/H, Ram.d., M.g./CP-11 and M.agr./C. with the indices 111.25-113.53, 12.72-13.03 and 11.67-11.86, respectively. We believe that these indices most fully characterize the resistance of the populations to drought and their high productivity under the stress (8.30-8.47 kg/m<sup>2</sup>). The Stress Tolerance Index (STI), with a range of 0.31 to 0.45, characterizes the ability of the genotype to form a stable yield regardless of stress factors. According to this index, there were eight populations that significantly surpassed the mean population, but they can be divided into two groups:

M.g./P.p., LR/H, Ram.d., M. g./CP-11 and M.agr./C. - STI index varies from 0.43 to 0.45 and they have a high yield under the stress of 8.30-8.47 kg/m<sup>2</sup> and mean under the irrigation - 19.22-20.44 kg/m<sup>2</sup>;

FHNV<sup>2</sup>, B11/P.d., Sin(s)/Prymorka, in which the STI index is 0.40, but high or moderate yield under the irrigation (19.5-20.65 kg/m<sup>2</sup>) and mean under the stress (7.26-7.76 kg/m<sup>2</sup>).

According to the relative drought resistance index (RDI), nine populations were identified: M.g./CP-11, Zymostiyka/M.K., A.r. d., M.g./M.agr., M.agr./C., Prymorka/Sin(s), LR/H, M.g./P.p., Ram.d. with a variation of the index from 1.06 to 1.14.

According to the drought resistance index (DI), there were eight populations that significantly exceeded the mean population, but they can be divided into two groups:

M.g./P.p., LR/H, Ram.d., M.g./CP-11 and M.agr./C. - DI index varies in the range of 0.45-0.49 and they have a high yield under the stress of 8.30-8.47 kg/m<sup>2</sup>;

Prymorka/Sin(s), B.11/P.d, Sin(s)/Prymorka, Zymostiyka/M.K. and M.g./M.agr., in which the DI index is 0.43, but low yields under irrigation (17.95-18.65 kg/m<sup>2</sup>) and average under the stress (7.60-7.76 kg/m<sup>2</sup>).

Studying the Modified Stress Tolerance Indices (M1STI, M2STI), eight (Ram.d., M.agr./C., B11/P.d., LR/H, Sin(s)/Prymorka, M.g./P.p., M.g./CP-11, Elehiya), with an M1STI index of 0.42-0.47 and five populations of alfalfa (M.agr./C., LR/H, Ram.d., M.g./P.p., Elehiya) with an index of M2STI – 0.54-0.57 were distinguished. The M1STI index is not always suitable for the selection of populations for drought resistance, as the selected populations are Sin(s)/Prymorka and B11/P.d. had high rates of 0.44-0.46 due to high yields under the irrigation (20.65; 20.36 kg/m<sup>2</sup>), but low or moderate under the stress (7.26; 7.52 kg/m<sup>2</sup>). The M2STI index more accurately characterizes drought-resistant genotypes that have high performance under the stress.

The Abiotic Tolerance Index (ATI) ranges from 57.77 to 67.95, in our case, it is not suitable for characterizing the resistance of alfalfa populations to stress, as, using this index, five populations were identified. (A.N.d. №15, M.g./CP-11, B11/P.d., Sin(s)/Prymorka, Elehiya), of which the last three had a high yield in the optimal conditions (20.36-20.65 kg/m<sup>2</sup>), but medium or low under the stress (6.66-7.52) and only one population M.g./CP-11 can be classified as drought-resistant with an ATI index of 60.67.

Based on the results of the study and their analysis, we proposed the stress resistance index ISR, which in our opinion characterizes the genotypes by the stress resistance not only by a smaller difference in the yield in the optimal and limiting conditions, but also considers high stress productivity.

Stress Resistance Index ISR is determined by the formula:

$$ISR = \frac{Y_p \times Y_s}{(Y_p - Y_s) \times (1 - \frac{Y_s}{Y_p})} \quad (15)$$

According to the Stress Resistance Index (ISR), nine populations were identified that significantly surpassed than the mean population, but they can be divided into two groups:

M.g./P.p., LR/H, Ram.d., M.g./CP-11 and M.agr./C. - ISR index varies between 23.53-26.67 and they have a high yield under the stress of 8.30-8.47 kg/m<sup>2</sup>;

Prymorka/Sin(s), Zymostiyka/MK, A.r.d. and M.g./M.agr., in which the ISR index is 22.29-22.86, but low yields under the irrigation (17.95-18.65 kg/m<sup>2</sup>) and average under the stress (7.60-7.76 kg/m<sup>2</sup>).

Yields of alfalfa populations under the stress (Y<sub>s</sub>) have a high positive correlation (r = 0.901-1.000) with indices YSI, YI, GMP, HMP, STI, DI, RDI, ISR and negative with SSI (r = -0.901); with the indices MPI, TOL, SSPI - the average correlation (r = 0.627; r = -0.609; r = -0.609), and with the ATI index there is no connection (r = -0.092).

## CONCLUSIONS

Analyzing alfalfa populations by forage productivity by the largest number of indices (11-13), we selected five genotypes: M.g./P.p., LR/H, Ram.d., M.g./CP-11 and M.agr./C., which had the highest yield of 8.30-8.47 kg/m<sup>2</sup> under the stress. Four indices YI, GMP, HMP, SSI and the proposed ISR stress resistance index were selected, which not only characterize the population in terms of drought resistance, but also in terms of productivity under the stress conditions.

Table 1. Drought tolerance - sown in 2017-2019 (total for two years)

Name	Abbreviation	Yp	Ys	MP	SSI	TOL	YSI	YI	STI	GMP	RDI	DI	SSPI	MiSTI	M <sub>2</sub> STI	ATI	HMP	ISR
Unintro, St.	G1	19.65	7.26	13.46	1.02	12.39	0.37	97.31	0.38	11.94	0.96	0.36	31.86	0.39	0.36	56.78	10.60	18.26
Elehiya	G2	21.47	6.66	14.07	1.12	14.81	0.31	89.27	0.38	11.96	0.81	0.28	38.08	0.46	0.30	67.95	10.17	14.00
Pymorka	G3	18.88	6.54	12.71	1.06	12.34	0.35	87.66	0.33	11.11	0.90	0.30	31.73	0.31	0.25	52.61	9.71	15.31
M.g./Pp.	G4	19.96	8.44	14.20	0.94	11.52	0.42	113.13	0.45	12.98	1.10	0.48	29.62	0.47	0.57	67.30	11.86	25.34
Sin (s)/Pymorka	G5	20.65	7.26	13.96	1.05	13.39	0.35	97.31	0.40	12.24	0.92	0.34	34.43	0.45	0.38	62.90	10.74	17.27
LR/H	G6	19.70	8.47	14.09	1.02	11.23	0.43	113.53	0.44	12.92	1.12	0.49	28.88	0.45	0.57	55.66	11.85	26.06
Pymorka / Sin(s)	G7	18.65	7.76	13.21	0.95	10.89	0.42	104.02	0.38	12.03	1.08	0.43	28.00	0.35	0.41	50.27	10.96	22.76
A.-N. d. № 114	G8	19.64	6.05	12.85	1.12	13.59	0.31	81.09	0.31	10.90	0.80	0.25	34.95	0.32	0.21	56.84	9.25	12.64
A.-N.d. № 15	G9	19.82	6.82	13.32	1.06	13.00	0.34	91.42	0.36	11.63	0.90	0.31	33.43	0.37	0.30	57.99	10.15	15.85
A.-N. d. № 38	G10	19.48	7.28	13.38	1.02	12.20	0.37	97.58	0.38	11.91	0.97	0.36	31.37	0.38	0.36	55.74	10.60	18.56
Dobir.k.s..	G11	18.05	6.53	12.29	1.04	11.52	0.36	87.53	0.31	10.86	0.94	0.32	29.62	0.27	0.24	47.99	9.59	16.03
Ram. d.	G12	19.22	8.42	13.82	0.91	10.80	0.44	112.86	0.43	12.72	1.14	0.49	27.77	0.42	0.55	52.71	11.71	26.67
(Emeraude / T.) <sup>2</sup>	G13	19.56	7.65	13.61	0.99	11.91	0.39	102.54	0.40	12.23	1.02	0.40	30.63	0.40	0.42	55.90	11.00	20.63
T./Emeraude	G14	19.60	7.21	13.41	1.03	12.39	0.37	96.64	0.37	11.89	0.96	0.36	31.86	0.38	0.35	56.51	10.54	18.04
M.g. CP-11	G15	20.44	8.30	14.37	0.96	12.14	0.41	111.25	0.45	13.03	1.06	0.45	31.22	0.50	0.56	60.67	11.81	23.53
Zymostiyka/M.K.	G16	18.65	7.72	13.19	0.95	10.93	0.41	103.48	0.38	12.00	1.08	0.43	28.11	0.35	0.41	50.32	10.92	22.48
M.agr/C.	G17	19.48	8.33	13.91	0.93	11.15	0.43	111.66	0.43	12.74	1.11	0.48	28.67	0.43	0.54	54.50	11.67	25.43
A.r. d.	G18	18.39	7.63	13.01	0.95	10.76	0.41	102.27	0.37	11.85	1.08	0.42	27.67	0.33	0.39	48.90	10.79	22.29
M.g./M.agr.	G19	17.95	7.60	12.78	0.94	10.35	0.42	101.87	0.36	11.68	1.10	0.43	26.61	0.31	0.37	46.38	10.68	22.86
M.g. d.	G20	18.62	7.18	12.90	1.00	11.44	0.39	96.24	0.35	11.56	1.01	0.37	29.42	0.32	0.33	50.75	10.36	19.02
FHN <sup>2</sup>	G21	19.50	7.76	13.63	0.98	11.74	0.40	104.02	0.40	12.30	1.04	0.41	30.19	0.40	0.43	55.41	11.10	21.41
V.11/P. d.	G22	20.36	7.52	13.94	1.02	12.84	0.37	100.80	0.40	12.37	0.96	0.37	33.02	0.44	0.41	60.96	10.98	18.91
Zh./CP-11	G23	19.76	7.29	13.53	1.02	12.47	0.37	97.72	0.38	12.00	0.96	0.36	32.07	0.39	0.36	57.42	10.65	18.30
Sybir. 8. d..	G24	19.18	7.37	13.28	1.00	11.81	0.38	98.79	0.37	11.89	1.00	0.38	30.37	0.36	0.36	53.87	10.65	19.44
<b>Mean by the population</b>		<b>19.44</b>	<b>7.46</b>	<b>13.45</b>	<b>1.00</b>	<b>11.98</b>	<b>0.38</b>	<b>100.00</b>	<b>0.38</b>	<b>12.03</b>	<b>1.00</b>	<b>0.39</b>	<b>30.82</b>	<b>0.39</b>	<b>0.39</b>	<b>55.27</b>	<b>10.76</b>	<b>20.04</b>
<b>Mediana</b>		<b>19.53</b>	<b>7.44</b>	<b>13.43</b>	<b>1.00</b>	<b>11.86</b>	<b>0.39</b>	<b>99.79</b>	<b>0.38</b>	<b>11.98</b>	<b>1.01</b>	<b>0.38</b>	<b>30.50</b>	<b>0.39</b>	<b>0.38</b>	<b>55.70</b>	<b>10.71</b>	<b>19.23</b>
LSD <sub>05</sub>		0.386	0.304	0.248	0.027	0.488	0.017	4.078	0.018	0.276	0.044	0.031	1.254	0.028	0.048	2.312	0.327	1.818
V, %		4.26	8.72	3.95	5.86	8.71	9.46	8.72	10.01	4.92	9.42	17.39	8.71	15.36	26.28	8.95	6.51	19.41
Sx		0.169	0.133	0.108	0.012	0.213	0.007	1.781	0.008	0.121	0.019	0.014	0.548	0.012	0.021	1.010	0.143	0.794

Table 2. Drought tolerance - sown in 2017-2019 (total for two years)

	Y <sub>p</sub>	Y <sub>s</sub>	MP	SSI	TOL	YSI	YI	STI	GMP	RDI	DI	SSPI	M <sub>1</sub> STI	M <sub>2</sub> STI	ATI	HMP	ISR
Y <sub>p</sub>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Y <sub>s</sub>	0.018	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MP	0.790	0.627	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SSI	0.416	-0.901	-0.228	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TOL	0.782	-0.609	0.236	0.891	-	-	-	-	-	-	-	-	-	-	-	-	-
YSI	-0.416	0.901	0.228	-1.000	-0.891	-	-	-	-	-	-	-	-	-	-	-	-
YI	0.018	1.000	0.627	-0.901	-0.609	0.901	-	-	-	-	-	-	-	-	-	-	-
STI	0.437	0.907	0.896	-0.633	-0.219	0.633	0.907	-	-	-	-	-	-	-	-	-	-
GMP	0.439	0.906	0.897	-0.633	-0.217	0.633	0.906	1.000	-	-	-	-	-	-	-	-	-
RDI	-0.416	0.901	0.228	-1.000	-0.891	1.000	0.901	0.633	0.633	-	-	-	-	-	-	-	-
DI	-0.202	0.974	0.439	-0.973	-0.768	0.973	0.974	0.789	0.788	0.973	-	-	-	-	-	-	-
SSPI	0.782	-0.609	0.236	0.891	1.000	-0.891	-0.609	-0.219	-0.217	-0.891	-0.768	-	-	-	-	-	-
M <sub>1</sub> STI	0.821	0.583	0.996	-0.173	0.288	0.173	0.583	0.871	0.870	0.173	0.388	0.288	-	-	-	-	-
M <sub>2</sub> STI	0.178	0.981	0.740	-0.811	-0.470	0.811	0.981	0.958	0.955	0.811	0.923	-0.470	0.704	-	-	-	-
ATI	0.993	-0.092	0.717	0.513	0.845	-0.513	-0.092	0.335	0.337	-0.513	-0.311	0.845	0.753	0.065	-	-	-
HMP	0.187	0.985	0.749	-0.814	-0.466	0.814	0.985	0.965	0.965	0.814	0.920	-0.466	0.711	0.991	0.078	-	-
ISR	-0.213	0.967	0.427	-0.973	-0.772	0.973	0.967	0.778	0.776	0.973	0.998	-0.772	0.375	0.917	-0.323	0.910	-

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