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RESEARCH ON THE EVOLUTION OF THE MORPHOLOGICAL, PHYSICAL, HYDRO-PHYSICAL PROPERTIES OF CHROMIC LUVISOIL FROM DOLJ COUNTY, IN THE PERIOD 1995-2021

Jenica CĂLINA, Aurel CĂLINA, Alin CROITORU

University of Craiova, Faculty of Agronomy, 19 Libertatii Street, Craiova, Romania

Corresponding author email: croitorualinc@yahoo.com

Abstract

The paper presents an original and multidisciplinary theme regarding the evolution of the main agro-productive properties given by the morphological, physical and hydro-physical properties of the chromic luvisols from Dolj County, exploited in the specific cultivation conditions of agritourism households. The novelty elements of this theme consist primarily in the tracking of all these properties over a long period of more than 25 years, using as a comparison term the chromic luvisol evolved under natural conditions, under forest vegetation. In order to be able to compare and observe the evolution of these properties over time, soil profiles were also carried out on the soils taken in cultivation, both in 1995 and in 2021, tracking their impact on the quality and quantity of agricultural productions, by significantly decreasing natural fertility and worsening these properties. Based on the significant and relevant results obtained, over a quarter of a century of research, it was concluded that this type of soil has undergone obvious changes in the sense of worsening some agro-productive properties, especially on cultivated soils.

Key words: *morphological, physical, hydro-physical properties, soil profile, soil pollution.*

INTRODUCTION

Soil is the most important resource of our planet, forming the base of our existence on Earth. Soil faithfully mirrors the influence of the anthropogenic activities (Zajícová & Chuman, 2019), climate, flora and fauna in variable periods of time (Hole, 1981).

Soil fertility, which offers continually and simultaneously the nutritive elements and water necessary for the growth and development of plant life, represents the guarantee of ensuring abundant, stable and healthy products (Patzel et al., 2000; Hansen et al., 2006), necessary to the generations of today and to those of tomorrow (O'Sullivan et al., 2017).

Identifying and understanding the temporal and spatial changes in the state of soil are essential for elaborating and implementing policies for the sustainable use of soil (Davidson, 2000; Várallyay, 2010; Smith, 2012; Bouma et al., 2017; Rhodes, 2017; Rahman & Singh, 2019), and also to have the certainty that the soil can ensure the continuity of goods and services (Doran & Zeiss, 2000; European Commission, 2006; Toth et al., 2016).

There is no doubt that there are strong connections between soil and human health, as recent studies explore our knowledge in this area (Brevik, 2013; Pepper, 2013; Rodrigues & Römkens, 2018; Zhou et al., 2019) and offer significant opinions regarding future research for soil and human health (Heckman, 2013; Bünemann et al., 2018; Brevik et al., 2020).

This article systematically examines the qualitative and quantitative characteristics chromic luvisols from the South-West region of Romania, utilized in the specific cultures of agritouristic farms and households. In Romania, agritourist households appeared after the fall of communism, as agritourism became a viable economic activity for small owners from the countryside. The seamless implementation of security for the offered services and traceability of the agri-food products in the agritourism activity represents a yet unreached milestone for Romanian owners of the small family farms, as it necessitates technical, preventative and self-controlled analyses (Mihalache et al., 2014; Tudor, 2019).

We considered how the data regarding the evolution of soil can contribute to establish preventative measures for negative phenomena.

The specific contribution of this paper is to consider this type of soil in relation to human health and how soil can be evaluated in the context of food security, namely agri-food quality and traceability.

In this work, only the evolution of the main morpho-physical-hydric properties of this type of soil is presented, because due to the duration of more than 25 years, during which the research was carried out, a lot of data and results were accumulated and we considered that the aspects related to the main chemical properties and soil contamination with heavy metals, to be presented in another paper, which will be published later.

MATERIALS AND METHODS

In order to achieve the stated objectives, field research was necessary, during which several soil profiles were morphologically analysed, and samples were collected from the most representative soils for laboratory analysis (Călina & Călina, 2019; Călina et al., 2022). Field maps and mapping works from the Dolj County Offices of Pedological and Agri-chemistry Studies were utilized. The soil profiles were taken from a depth of about 200 cm. Each profile was morphologically characterized, with the following properties being established: thickness of the horizons, color, texture, structure, porosity, compactness, adhesion, neoformation, moisture, and parent rock. Soil samples were collected from every layer of the profiles distributed by soil genesis, replicated 3 times, in its undisturbed and disturbed structure, using cylinders (5 x 5 cm).

Soil profiles have the following particularities:

Profile under forest vegetation: location - 4 km North-West from Șimnic in Tufarul Viilor forest; altitude - 170 m average height; relief - piedmont plateau Leu-Rotunda, flat terrain; vegetation - Querce forest formed by: *Quercus freinetto, Quercus cerris, Quercus pubescents, Acer campestre, Crataegus monogina, Ulmus* sp.; parent material - clay deposits combined with loess deposits; natural drainage - good; groundwater depth - more than 15 m.

Profiles under agricultural corps: location - 3 km North-Est from Șimnic; altitude - 168 m average height; relief - piedmont plateau Leu-Rotunda, parent material - marly deposits and clayey marls, natural drainage - not good; groundwater depth - more than 16 m.

Soil profiles were taken in approximately the same place, 25 years apart, in the same climatic, relief, fauna, and anthropic conditions, thus the same paedogenetic conditions.

a) ***Determination of physical and mechanical properties***

In order to determine the physical properties of soils, the samples collected in their undisturbed structure (in metal cylinders) were analysed as follows: *particle size distribution* - Kachinsky method; the texture - by the Chiriţă-Burt triangle; *soil density* (D, g/cm^3) - pycnometer method; *bulk density* (BD, g/cm^3) - the National Research and Development Institute for Soil Science, Agri-chemistry and Environment method, by reference to the dry soil mass in the oven at the volume of the cylinder in which the soil sample was taken; *resistance to penetration* (RP, kgf/cm^2) - method of resistance to dynamic penetration, on samples taken in metal cylinders, the soil being brought to a moisture equal to 50% of its capillary water capacity (Canarache, 1990; Dumitru et al., 2011; Stătescu et al., 2013).

total porosity (Pt, %) - calculated with Eq. (1): $Pt\ (\%) = 100 \cdot (1 - \frac{Da}{D})$ (1)

air porosity (Pa, %) - calculated with Eq. (2): $Pa = Pt - Fc \cdot BD$ (2)

b) ***Determination of hydro-physical properties:***

- *water permeability* - the National Research and Development Institute for Soil Science, Agri-chemistry and Environment method; hygroscopicity coefficient (HC, %) - Mitscherlich method; wilting coefficient (WC, %) - through calculation using Eq. (3): $WC = HC \cdot 1.5$ (3);
- *the moisture equivalent* (ME, %) - by centrifugation of soil samples with a force greater than 1,000 times the gravitational acceleration;
- *field capacity* (FC, %) - calculated using Eq. (4): $FC = ME \cdot 0.84 + 2.64$ (4);
- *available water capacity* (AWC, %)-through calculation, using the formula Eq. (5):

$$AWC = FC - WC \quad (5);$$

- *hydraulic conductivity/permeability* (K, mm/h) - under laboratory conditions by infiltration under constant degree - National Research and Development Institute for Soil Science, Agri-chemistry and Environment (ICPA) Bucharest.

RESULTS AND DISCUSIONS

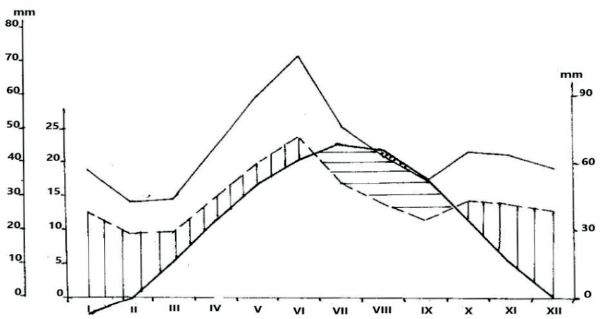
Characterization of the researched area

The chromic luvisols in Oltenia it occupies a strip defined by the North by Turnu-Severin, Bistrita, Terpezita, Ghercești, Bals, Piatra-Olt, and in the South by Burila Mare, Braniște, Mărăcinele, Segarcea, Tâmburești, Deveselu and Caracal. *Chromic luvisols* are defined by the presence of the El and Bt horizons and formed in the wetter part, north of the researched area. These soils are found in a complex with other chromic luvisols, occupying in the researched area approximately 10,283.28 ha, 7.4% respectively of the total *chromic luvisols* soils in this area (Florea et al., 1987; Stănilă, 2016).

The relief is represented by flat surfaces and depressions, such as the Getic Piedmont, the high Leu-Rotunda field and the high terraces of the Jiu, where rainwater accumulates due to the impermeable soil layer, leading to the phenomenon of podzolization. The average altitude of the relief is about 170 m. From a geological point of view, the area with chromic luvisols falls in the Upper Pleistocene (Coteț, 1973).

The parent material on which it was formed is generally composed less often of loess deposits, with the predominance of marly deposits and marly clays which hinder the internal drainage capabilities of the soil (Șorop & Vasile, 1990).

The climate of this area according to Kopper falls mostly under Cfax type and to a lesser extent under Cfbx. The climatic conditions in the area of *chromic luvisols* soils in Oltenia contributed greatly to the formation of these types of soils, which have different properties from other types existing in our country. Characterized on the basis of meteorological data from Craiova station, it falls within the continental type with a weak Mediterranean influence and has the following Cfax formula, according to Köper (Șorop & Vasile, 1990; Mihalache, 2006).



average monthly temperature; 1/2 scale precipitation; 1/3 scale precipitation; wet period; drought period; dry period; excessively wet period; P- the average amount of annual rainfall; Pv- the average amount of rainfall in the growing season; T- multiannual average temperature.

Figure 1. Climateogram type Walter H. - Lieht H., by the Craiova meteorological station, multiannual average (1970-2021)

The average annual temperature according to Craiova Meteorological Station is 10.8°C, and the number of days without frost is 102. The average number of days with frost is about 36 days. The duration of the frost-free interval is 203 days, which influences the duration of the vegetation period and agricultural crops. The average number of frost days in Craiova is 102 days (Păunescu, 1975). The average amount of annual precipitation is about 500-550 mm, and evapotranspiration is about 685 mm (Figure 1). The annual sum of temperatures higher than 0°C amounts to 4200°C, which determines good conditions for the growth and development of many crops.

Hydrography and hydrogeology have a special importance in the soil formation process. Of greater importance for the paedogenetic process are the surface waters (rivers) that can sometimes overflow, the groundwater in terms of depth, degree and type of mineralization, coastal springs, stagnant surface waters (from precipitation) that condition the pseudo-gleization process (Păunescu, 1975).

From a hydrographic point of view, the area of *chromic luvisols* in the researched area is drained by two large rivers, the Jiu which borders the western area and the Olt which represents the eastern border. The Jiu is characterized by a valley 120 m deep in Craiova, and 80 m in Rojiște, compared to the level of the fields that border it. The Olt is the second basic component of the hydrographic network in this area. On its left side its most important tributaries are: Dârjov and Iminog. On the right side the tributary valleys are formed by: Olteț, Teslui, and Caracal valleys, and on the western side, the Buca and Arțăroasa tributaries, whose valleys have parallel confluences (Coteț, 1973).

Vegetation. The monotony of the landscape and the low altitudes determine a relative uniformity of the vegetation. In most parts of the territory, natural vegetation has been replaced by agricultural crops. The natural vegetation encountered on smaller areas in the valleys comes in the form of clusters of forests (e. g. Tufarul Viilor), small areas of grassland and poor-quality meadows or as weeds in agricultural crops. The species of wood plants are *Quercus pubescens*, *Quercus pedunculiflora, Fraxinus excelsior*, and the brushwood is represented by the wild privet, the blackthorn, the hawthorn and the rose (Răduțoiu et al., 2018).

Among the most common grassy species we can find meadow fescue, bluegrass, rape field, couch grass, thick knot grass, amaranth, bindweed, foxtail, thistle, chicory, red poppy, plantain and thistle. The depression areas where the precipitation water stagnates on the surface, herbaceous vegetation replaces the forest and is represented by: *Gratiola officinalis, Grafolium uliginosum, Phragnites australis, Scirpus lacustris, Typha latifolia* and *Trifolium arvense* (Răduțoiu et al., 2018).

Agri-pedological characterization of chromic luvisols

a) ***Morphological characteristics***

The comparative analysis of the morphological characteristics (Table 1) highlights a profile with clearly defined horizons, in the case of the chromic luvisol that evolved under the influence of forest vegetation and the intervention of the anthropic factor through agriculture, constantly present on the surface horizons of the soils that evolved on the lands used for cultivating crops in the agritourist farms (horizon Ao 29 cm in 1995 and 33 cm in 2021).

Table 1. Morphological characteristics of soil profiles of the chromic luvisol

Profile layer / Depth (cm)	***Morphological characteristics***
Year - 1995 - Forest vegetation	
Ao horizon: 0-22 cm	Dark brown-gray color (10YR 5/3) in wet state and (10YR 5/4) in dry state; clay texture; medium and small glomerular structure, well developed; crumbly when wet and hard when dry; weak plastic; weak adhesive; moderately compact; frequent earthworm channels containing coprogen agglomerations; frequent grassy roots and rare small woody roots; gradual transition.
AB horizon: 22-33 cm	Dark brown-gray (10YR 4/2) in wet state and light gray-brown (10YR 6/2) in dry state; clay texture; medium and small polyhedral subangular structure, moderately developed; friable in wet state and harder in dry; weak plastic; weak adhesive; moderately compact; rare earthworm channels containing coprogen agglomerates; large and sparse grassy roots and a thick woody root at the base; gradual transition.
BE horizon: 33-45 cm	Dark brown-gray color (7.5YR 4/2) in wet state and (7.5YR 5/3) in dry state; clay texture; polyhedric subangular and medium and large angular structure, poorly developed; crumbly when wet and harder when dry; quartz powder on the surface of structural aggregates and quartz accumulations in the form of whitish spots; non-plastic; non-adhesive; weak compact; a large woody root at the top and several small, sparse grassy roots; very rare earthworm channels containing coprogen agglomerates; gradual transition.
Bt_1 horizon: 45-96 cm	Reddish-brown-gray (7.5YR 4/3) when wet and (7.5YR 5/3) when dry; clay loam texture; medium and large prismatic structure, well developed; firmly moist and hard dry; almost continuous clay film on horizontal and vertical structural faces; plastic; adhesive; compact; ferro-manganous punctate separations and small and rare grains; frequent medium and thin woody roots especially in the upper part of the horizon; root channels; gradual transition.
Bt_2 horizon: 96-149 cm	Dark reddish brown (7.5YR 4/4) when wet and (7.5YR 5/4) when dry; clay loam texture; medium and large prismatic structure, well developed; firmly when moist and hard when dry; plastic; adhesive; compact; discontinuous film of clay on vertical and horizontal structural faces; relatively frequent small ferro-manganous and grain repairs; very rare thin and medium roots; root channels; gradual transition.

Bt_3 horizon: 149-200 cm	Yellowish-reddish brown (7.5YR 5/4) when wet and (7.5YR 6/4) when dry; clay loam texture; prismatic structure, poorly developed; plastic; adhesive; compact; clay film in the form of spots and vines; fine pores; weak manganese grains.
Year - 1995 - Agriculture crop	
Ao horizon: 0-29 cm	Brown-gray color (7.5YR 4/3) in wet state; very poorly developed prismatic structure; clay texture; frequent roots; loose; weak compact; damp; gradual transition.
AE horizon: 29-44 cm	Slightly yellowish brown-gray color (7.5YR 3/2); colloidal silica powder; clay texture; poorly developed prismatic structure; compact; rare thin roots; gradual transition.
Bt_1 horizon: 44-73 cm	Brown color with reddish tinge (10YR 5/3); clay loam texture; medium and poorly developed subangular polyhedral structure; damp; common manganese grains; gradual transition.
Bt_2 horizon: 73-108 cm	Dark brown with reddish tinge (10YR 4/3); clay loam texture; large polyhedral structure; compact; damp; fine pores; common manganese grains; contains traces of roots; gradual and linear transition.
Bt_3 horizon: 108-141 cm	Brown color with reddish tinge (10YR 5/4); clay loam texture; large and poorly developed subangular polyhedral structure; dense manganese spikes; compact; damp; weak traces of roots are noticed; gradual transition.
BC horizon: 141-200 cm	Reddish-yellowish brown (10YR 4/3); clay loam texture; large subangular polyhedral structure; very compact; without roots; damp; very low concentrations of calcium carbonate at the base.
Year - 2021 - Agriculture crop	
Ao horizon: 0-33 cm	Dark brown-gray color (10YR 3/2) in wet state and (10YR 4/3) in dry state; clay loam texture; large, well-developed granular and grainy structure; plastic; adhesive; weak compact; fine and frequent pores; gradual transition.
EB horizon: 33-71 cm	Dark reddish brown-gray color (7.5YR 3/2) in wet state and (7.5YR 4/2) in dry state; clay loam texture; subangular and angular polyhedral structure; weak adhesive; moderately compact; common manganese grain; weak powdering of quartz on the surface of aggregates and weak accumulations of quartz in the form of whitish spots; gradual transition.
Bt_1 horizon: 71-102 cm	Dark reddish-brown color (7.5YR 3/2) in wet state and (7.5YR 4/3) in dry state; clay loam texture; large and medium prismatic structure; plastic; adhesive; moderately compact; almost continuous clay films on the surface of structural aggregates; frequent small grains; gradual transition.
Bt_2 horizon: 102-140 cm	Slightly dark yellowish-reddish brown (7.5YR 4/3) in wet state and (7.5YR 4/4) in dry state; clay loam texture; medium and large prismatic structure; plastic; compact; continuous clay film on the surfaces of structural aggregates; frequent and medium-sized small grains; leaks of darker material from the upper horizons; gradual transition.
Bt_3 horizon: 140-171 cm	Dark yellow-reddish brown (7.5YR 4/3.5) in wet state and (7.5YR 5/4) in dry state; clay loam texture; small, moderately developed angular and prismatic polyhedral structure; plastic; adhesive; compact; clay film in the form of stains; frequent small grains; gradual transition.
BC horizon: 171-200 cm	Dark reddish brown-yellowish color (7.5YR 4/4) in wet state and (7.5YR 6/5) in dry state; very poorly developed prismatic structure; small and frequent manganese grains; clay film in the form of stains; small and very rare veins, spots and concretions of $CaCO_3$.

Source: own results

From a morphological stand point, significant modification can be observed in the thickness of the Ao and EB horizons, for the soils utilized for agriculture, this change being more evident in the year 2021. Also, in the year 2021 the leaching process manifests up to a depth of 71 cm (Figure 2, Table 1).

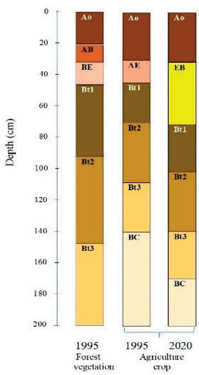


Figure 2. Soil profiles by vegetation types and over time

b) ***Granulometric composition***

The chromic luvisol soil developed under woody vegetation has a coarse sand content of around 10%, with slight decreases in the B horizons, up to 7.5%.

The percentage of fine sand is higher than the course one, registering a slight decrease in profile from 37.9%, in the Ao horizon, to 25.1% in the Bt_2 horizon.

The dust fraction has values around 20%, with slight increases and decreases from one horizon to another. The clay registers average values with increases per profile from 30.4% at the surface, to 42.7% in the Bt_2 horizon. This particle size composition imprints a loamy texture on the soil in the first horizons and clay-loamy and loam-clay in the following horizons (Table 2).

The cultivated chromic soil luvisol has, from a granulometric point of view, a coarse sand content higher than 8.5%, in the first horizons, which decreases slightly in the deep horizons, up to 6.3%, in Bt_4. Fine sand registers a percentage of 37.5% in the Ao horizon and decreases to 26.2% in Bt_1. Adding these two fractions of sand, it was remarked that they approach 50% in the first 3 horizons and fall below 40% in the next 4 horizons. The fine fraction, clay registers a lower percentage in the horizon Ao (29.2%) and increases strongly at the level of B horizons, reaching 47.8% in Bt_1 (Table 2). This granulometric composition imprints the soil a loamy texture in the first 2 horizons, clay-loamy in Bt_1 and Bt_2 and loam-clayey in the last 2 horizons.

Table 2. Particle size distribution of the chromic luvisol

Horizon	*Horizon depth (cm)*	*Sampling depth (cm)*	*Particle size distribution (%)*				*Textural class*
			Coarse sand 2-0.2 mm	*Fine sand 0.2-0.02 mm*	*Silt 0.02-0.002 mm*	*Clay <0.002 mm*	
Year - 1995 - Forest vegetation							
Ao	0-22	5 - 15	10.4	37.9	21.3	30.4	Loam
AB	22-33	22 - 32	9.6	36.1	22.7	31.6	Loam
BE	33-45	35 - 45	8.8	35.8	24.9	30.5	Loam
Bt_1	45-96	70 - 80	7.5	34.9	19.1	38.5	Loam clay
Bt_2	96-149	120 - 130	10.9	25.1	21.3	42.7	Loam clay
Bt_3	149-200	175 - 185	10.2	28.4	24.1	37.3	Loam clay
Year - 1995 - Agricultural crop							
Ao	0-29	10 - 20	10.31	27.87	30.69	31.13	Loam
AE	29-44	30 - 40	10.74	27.41	27.91	33.94	Loam clay
Bt_1	44-73	50 - 60	8.76	25.44	27.98	37.82	Loam clay
Bt_2	73-108	85 - 95	7.41	22.08	24.27	46.24	Clay loam
Bt_3	108-141	120 - 130	8.19	26.83	27.85	37.13	Loam clay
BC	141-200	175 - 185	14.21	23.48	28.76	33.55	Loam clay
Year - 2021 - Agricultural crop							
Ao	0-33	10-20	8.5	37.5	24.8	29.2	Loam
EB	33-71	50-60	8.5	35.8	25.3	30.4	Loam
Bt_1	71-102	85-95	5.6	26.2	20.4	47.8	Clay loam
Bt_2	102-140	115-125	6.9	28.7	19.3	45.1	Clay loam
Bt_3	140-171	150-160	6.3	31.3	19.9	42.5	Loam clay
BC	171-200	180-190	6.3	32.1	19.4	42.2	Loam clay

Source: own results

c) ***The main physical properties***

Regarding the main physical properties, the chromic soil luvisol developed under woody vegetation is looser at the surface, compared to the cultivated soils, thus, the apparent density (AD) has the value of 1.24 g/cm^3 in the horizon Ao and 1.46 g/cm^3 in the transition horizon BE. The total porosity (Pt) has a value of 51.4%, in Ao and 43.56% in the BE horizon, and the aeration porosity (Pa) is 19.8%, in the first horizon and 12.24% in the second. In the deep horizons the values of the physical properties of the soil increase or decrease, approaching those of the cultivated soils, which demonstrates that the influence of the anthropogenic factor is felt only in the first half of the soil profile (Table 3)

Comparatively, in the chromic soil luvisol evolved under crop culture, there is a strong compaction, the apparent density (DA) increasing from 1.37 g/cm^3 (Ao) to 1.58 g/cm^3 (Bt_3). The compaction is also highlighted by the total porosity which decreases in profile from 47.5% to 42.3% with depth. Also, the resistance to penetration (RP) has high values in the Bt_2 horizon 119 kgf/cm^2 (Table 3).

Table 3. Main physical properties of the chromic luvisol

Horizon	*Horizon depth (cm)*	*Sampling depth (cm)*	*BD (g/cm^3)*	*D (g/cm^3)*	*Pt (%)*	*Pa (%)*	*RP (kg f/cm^2)*
Year - 1995 - Forest vegetation							
Ao	0-22	5 - 15	1.24	2.55	51.40	19.80	50
AB	22-33	22 - 32	1.36	2.60	47.70	12.24	52
BE	33-45	35 - 45	1.49	2.64	43.56	1.17	68
Bt_1	45-96	70 - 80	1.50	2.67	43.82	-	89
Bt_2	96-149	120 - 130	1.54	2.69	42.75	-	92
Bt_3	149-200	175 - 185	1.53	2.70	43.35	-	99
Year - 1995 - Agricultural crop							
Ao	0-29	10 - 20	1.21	2.58	53.10	27.80	45
AE	29-44	30 - 40	1.33	2.62	49.20	18.10	59
Bt_1	44-73	50 - 60	1.47	2.65	44.50	9.20	67
Bt_2	73-108	85 - 95	1.54	2.68	42.53	1.31	92
Bt_3	108-141	120 - 130	1.57	2.67	41.19	9.13	73
BC	141-200	175 - 185	1.60	2.67	40.07	8.29	69
Year - 2021 - Agricultural crop							
Ao	0-33	10-20	1.37	2.61	47.50	14.42	62
EB	33-71	50-60	1.46	2.66	45.11	12.27	71
Bt_1	71-102	85-95	1.48	2.69	45.00	4.50	114
Bt_2	102-140	115-125	1.54	2.71	43.20	3.28	119
Bt_3	140-171	150-160	1.58	2.73	42.10	1.87	86
BC	171-200	180-190	1.58	2.74	42.30	2.65	91

BD - Bulk density; D - Soil density; Pt - Total porosity; Pa - Air porosity; RP - Resistance to penetration; Source: own results

d) ***The main hydro-physical properties***

From table 4, we notice that in the soil under woody vegetation, the hydro-physical indices have values that correlate well with the granulometric composition, increasing from the surface to the B horizons, where the largest number of fine fractions accumulate. The high values of the main hydro-physical indices for this type of soil in the upper horizon, compared to those recorded in the soil utilized for cultivation of crops, are mainly due to the high content of organic matter.

Furthermore, from this table it can be noted that the hydro-physical indices from the chromic luvisol used for agriculture correlate well with the clay content of the soil, increasing at the level of the B horizons, where the accumulation of clay has the highest percentage. Thus, at the level of the Bt1 horizon, the wilting coefficient (WC) has the value of 16.45%, the field capacity (FC) 27.36%, and the water permeability 0.54 mm/h (Table 4).

Commenting on the data presented in Tables 1 to 4 regarding the values of the indices obtained for the soil profiles under woody vegetation and cultivated crops, we found that the apparent density (AD) for soil under woody vegetation is lower than for cultivated soil. The differentiation is significant, especially in the first horizons. The same changes in terms of use are observed in the porosity. Thus, in the chromic soil luvisol evolved under natural conditions of formation, under woody vegetation, the total porosity (Pt) and the aeration porosity (Pa) have high values in the first horizon, around 50% and around 20%, respectively. In the cultivated soil, the total porosity (Pt) decreases in the surface horizon to 47.50%, and the aeration porosity (Pa) decreases to 14.42%. Also, the resistance to penetration (RP) is less than 50 kgf/cm^2, in the Ao horizon, for the chromic soil luvisol under the forest and 62 kgf/cm^2, for the cultivated soil. Water permeability is better for the chromic soil luvisol under the forest, being 5.4 mm/h, compared to 3.0 mm/h for the cultivated soil. In the cultivated soil, this decreases a lot except for the surface horizon from 1995, when it is lower, due to the loosening of the soil through special agri-technical works.

Table 4. Main hydro-physical values of the chromic luvisol

Horizon	*Horizon depth (cm)*	*Sampling depth (cm)*	*HC (%)*	*WC (%)*	*ME (%)*	*FC (%)*	*AHR (%)*	*Permeability (mm/h)*
Year - 1995 - Forest vegetation								
Ao	0-22	5 - 15	7.26	10.89	26.48	25.52	14.63	5.48
AB	22-33	22 - 32	8.64	12.96	27.11	26.07	13.11	5.21
BE	33-45	35 - 45	9.27	13.90	29.84	28.43	14.52	2.56
Bt_1	45-96	70 - 80	11.49	17.23	33.67	31.68	14.44	0.98
Bt_2	96-149	120 - 130	12.60	18.90	34.35	32.33	13.43	0.52
Bt_3	149-200	175 - 185	8.24	12.11	28.81	24.92	12.81	0.62
	Year - 1995 - Agricultural crop							
Ao	0-29	10 - 20	5.47	8.20	21.18	20.94	12.73	6.21
AE	29-44	30 - 40	6.31	9.46	23.97	23.35	13.88	3.40
Bt_1	44-73	50 - 60	8.26	12.39	24.76	24.03	11.64	2.58
Bt_2	73-108	85 - 95	10.19	15.28	27.91	26.76	11.47	2.16
Bt_3	108-141	120 - 130	7.42	11.18	20.58	20.42	9.24	3.96
BC	141-200	175 - 185	6.04	9.06	19.94	19.86	10.80	4.78
	Year - 2021 - Agricultural crop							
Ao	0-33	10-20	7.30	10.95	23.76	23.17	12.22	3.01
EB	33-71	50-60	7.30	10.95	22.98	22.49	11.53	2.48
Bt_1	71-102	85-95	10.97	16.45	28.61	27.36	10.90	0.54
Bt_2	102-140	115-125	10.44	15.66	26.94	25.92	10.26	0.37
Bt_3	140-171	150-160	9.86	14.79	26.41	25.46	10.67	0.64
BC	171-200	180-190	9.83	14.74	25.98	25.09	10.34	0.21

HC - Coefficient of hygroscopicity; WC - Wilting coefficient; ME - Moisture equivalent; FC - Field capacity; AHR - Active humidity range; Source: own results

How it is used is also very significantly negative for some chemical properties, especially in terms of organic matter content. Thus, in the soils under woody vegetation the percentage of organic matter is high, 4.02%, and in the cultivated ones, the soil content in organic matter decreased almost by half, being in the first horizon of 2.35%.

Thus, we can state that by cultivating crops on the chromic soil luvisols in the researched area, they undergo more or less accentuated changes. Among these changes we mention a stronger settlement, a worsening of the porosity, a decrease of the permeability and the content of organic matter. Given these trends, measures must be taken to counteract them, such as a deep loosening and organic fertilization to increase porosity, improve the structure and increase the humus reserve in the soil.

CONCLUSIONS

Research on this type of soil on the main agri-productive properties has found that in terms of morphological characteristics, i.e., the succession and thickness of the horizons, there were no significant changes, both on the soil under woody vegetation and on soils used for cultivating, during the 25 years of observations. Regarding the granulometric composition, there is a decrease in the clay content in the cultivated soils, an increase of the clay content and an increase of the coarse fractions (sand), due to the washing phenomenon, the cultivated plants having a lower retention capacity of fine clay particles. This phenomenon is also present in depth where the amount of clay removed from the soil surface is deposited in the Bt horizons.

From the point of view of physical properties, there is a considerable decrease in the total porosity and aeration of the soil use for crop cultivation, compared to the soil developed under woody vegetation, especially in 2021, where aeration porosity reaches only 14.42%. Along with their decrease, there is a worsening of the soil aeration regime and a substantial increase in penetration resistance, by approximately 12 kgf/cm^2, in 2021, compared to that of the soil under woody vegetation.

At the hydro-physical level indices the cultivated soils, compared to the one under woody vegetation presents a decreased FC - field capacity and AHR - active humidity range, an aspect which is due mainly to the washing phenomenon that led to the elution of clay particles and organic matter from the surface layer, their retention at the soil surface being more effective on soils with permanent vegetation, such as forests. And permeability to water decreased by about two units, due primarily to subsidence of the soil surface layer as a result of repeated passages with heavy soil tillage equipment.

To sum up, it can be stated that from following the research, the main agri-productive properties of chromic luvisol have changed very significantly, natural fertility decreasing greatly on cultivated soils, compared to those developed in a natural state, under woody vegetation. It has also been highlighted that due to the cultivation technologies applied in agritourism farms, with a lower number of processing works and inputs of pesticides, fungicides and insecticides, the main agri-productive properties of the soil have been preserved even after an operating period of more than 25 years.

Following these conclusions, it is recommended that, for the total protection of the soils, crops and tourists, the agritourist farms in Romania must practice an organic agriculture that involves the application of agricultural technologies that are sustainable, diversified and balanced to ensure the preservation of the environment and a certain nutritional and traceable quality of the food served.

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ALLUVIAL SOILS OF THE LOWER DNIESTER MEADOW - GENETIC FEATURES AND CLASSIFICATION PRINCIPLES

Valerian CERBARI, Tamara LEAH

Institute of Soil Science, Agrochemistry and Soil Protection "Nicolae Dimo", 100 Ialoveni Street, 2070, Chisinau, Republic of Moldova

Corresponding author email: tamaraleah09@gmail.com

Abstract

In the Lower Dniester meadow from the Republic of Moldova are spread the following subtypes of alluvial soils: ochric (poorly evolved), typical, humic, slitizated, hydric, salinized, solonetzed, gleyic. The most widespread are the clayey-loamy humic alluvisols in the central meadow, the post-marshy clayey deep-humic alluvisols in the meadow under the terrace and the weak humiferous alluvisols with semi-deep humiferous profile and humiferous soil layers buried deeper than 70 cm, formed on the loamy-sandy grind near the bed of the Blind Dniester. Most of the alluvisols in the Lower Dniester meadow are irrigated or have been irrigated. The texture is one of the most spatially variable on the profile of the alluvial soils in the Lower Dniester meadow.

Key words*: alluvial soil, Lower Dniester, texture, classification, genetic peculiarities.*

INTRODUCTION

In each soil-climatic zone, the territories of river valleys are peculiar natural landscapes. Alluvial (floodplain) soils are widespread here, distinguished by their natural fertility and being the most valuable agricultural land (Leah, 2019; Leah & Cerbari, 2019).

Floodplain - part of the river valley, periodically flooded with hollow waters of the rivers. A feature of soil formation in the territory of floodplains, which determines many features of the genesis, composition and properties of alluvial soils, is the development of floodplain and alluvial processes. Floodplain processes - periodic flooding of the territory of the floodplain with hollow waters.

River floods are seasonal and are associated with spring snowmelt, spring-summer melting of glaciers, and heavy monsoon rains. Hollow waters can flood the floodplain from several hours to several weeks (1.5-2 months). This is a kind of natural irrigation of the floodplain. It has a great versatile effect on soil formation: it creates a different water regime than on non-floodplain soils, affects the level and composition of groundwater, softens the soil climate, and promotes the activation of microbiological and geochemical processes (Leah et al., 2019; Leah et al., 2019). All this affects the composition and productivity of natural vegetation, salt, biochemical and soil and groundwater regimes (Лях, 2020; Канаш, 2004; Наконечний & Позняк, 2011).

Alluvial deposits are the mineral base from which floodplain soils are created. Therefore, the composition, properties of alluvium, its thickness, and the frequency of deposition are of decisive importance for the genesis of soils. The nature of the alluvial process is influenced primarily by the position of individual parts of the floodplain in relation to the riverbed. The territory of the floodplain, depending on the distance from the river, is divided into three parts (Вильямс, 1955): near-river, central and near-terrace. They differ in the composition of alluvial deposits, relief, depth of groundwater and, as a result, in vegetation and soil cover.

MATERIALS AND METHODS

In the process of researching the soil cover of the Lower Dniester meadow, the methods approved in Moldova were used for carrying out pedological research in the field, laboratory and office. Soil profiles with a depth up to the groundwater were placed in the field. The groundwater level in the recently drained meadow varies from a depth of 2.5 m in the middle part of the meadow to 1.5 m - in the meadow near the river bed. For laboratory analyses, samples were taken from each genetic horizon of the soil profiles (Florea et al., 1987). The analyses were performed according to the standardized methods in force.

RESULTS AND DISCUSSIONS

The surface of the Lower Dniester meadow is raised above sea level by only 4-5 m, which ensures a slow division of alluvial deposits during overflows and, in the past, until the construction of dikes and the drainage system, the contribution to the swamping of large areas of land in the meadow. At the moment, the groundwater on the dry lands is lowered deeper than 2 m from the land surface and the meadow is characterized by a satisfactory water regime.

Most of the alluvial soils in the Lower Dniester meadow are irrigated or were irrigated in the past. Until 1990 these soils were used in vegetable cultivation. Unfortunately, this vegetable production complex has been mostly damaged, but it can be restored (Didenco, 2022; Leah & Cerbari, 2019; Leah, 2018).

In the Lower Dniester meadow, humic loamy-clay alluvial soils are spread from the central meadow (Profile A5); the deep humic loamy post-marshy alluviums from the meadow below the terrace (Profile A1 and 275) and the slightly humic alluviums with a semi-deep humic profile and layers of humic soil buried deeper than 70 cm, formed on the loamy-sandy gravel near the bed of the Blind Dniester (Profile A9).

Profile A9. Alluvisol low humiferous, semi-deep humic profile and humic soil layers buried deeper than 70 cm, loamy-sandy, moderately carbonate, arable, irrigated (Figure 1).

Profile A5. Alluvisol humiferous, very strongly deep stratified humic profile, loamy-clayey, moderately carbonatшc, arable, irrigated (Figure 2).

Profile A1. Alluvisol deep humiferous, very strongly humiferous profile, loamy, weak carbonatшc, arable, irrigated, post-marshy (Figure 3).

Profile 275. Alluvisol deep humiferous with a very strongly deep humic profile, weakly carbonatic, loamy, gleyed 62-130 cm, gleyic deeper than 130 cm, well-drained, post-marshy (Figure 4).

Granulometric composition of alluvium depends on the speed of hollow water movement along the floodplain: the greater the speed, the more stable fine (silty-dusty) particles in the flow, the larger the size of the settling particles.

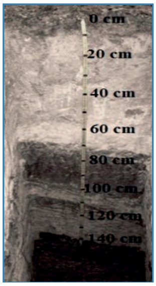

Figure 1. Profile A9


Figure 2. Profile A5

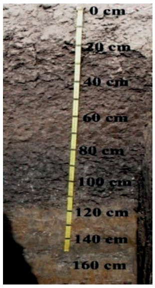

Figure 3. Profile A1

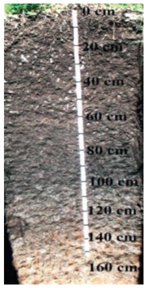

Figure 4. Profile 275

Since the velocity of hollow waters decreases with distance from the channel deep into the floodplain territory, the composition of alluvium gradually changes: predominantly loamy-sandy alluvium is deposited in the near-river part, and loamy-argillaceous sediments are deposited in the central and terraced parts.

Therefore, with distance from the river, the granulometric composition of alluvial soils also changes: the proportion of sandy particles in it decreases and the content of silty and silty particles increases. The latter always contain more organic matter and plant nutrients. Consequently, in the central and terraced floodplains, the soil-forming process develops on sediments richer in chemical composition and diverse in mineralogical than in the near-river part (Leah, 2018).

The texture or granulometric composition of the soil means the proportion in which soil particles of different sizes (from colloidal clay to coarse sand) participate in the composition of the soil.

The categories of particles of different sizes, which make up the solid phase of the soil, are called particle size fractions, which have been grouped into size classes, sand, dust and clay.

The proportion of these fractions in the soil determines the textural class of the soil.

Texture is one of the most important characteristics of alluvial soils and also one of the most variable spatially and on the soil profile in the Lower Dniester meadow.

Depending on the relief of the meadow, formed by the old beds of the Dniester, on the territory of the meadow there are both alluvial soils with a coarse loamy-sandy or loamy-sandy texture on the gravels near the riverbeds, as well as soils with a medium and fine texture on the territory of the central meadow and below the terraces - loamy-clay, clayey-loamy, clayey and fine clayey (Table 1).

Table 1. The texture of the Alluvisols in the Lower Dniester meadow

The horizon and depth, cm	The fractions size (mm); content (% g/g)							
	1.0-0.25	0.25-0.1	0.1-0.05	0.05-0.01	0.01-0.005	0.005-0.001	<0.001	<0.01
Profile A9. Alluvisol low humiferous with semi-deep humic profile and humic soil layers buried deeper than 70 cm, loamy-sandy, moderately carbonate, arable, irrigated								
0-20	0	0	30.2	36.4	5.6	10.6	17.2	33.4
20-40	0	0	29.0	36.4	8.8	10.1	15.7	34.6
40-58	0	0	26.8	42.1	5.7	11.4	14.0	31.1
58-71	0	0	25.7	45.2	6.6	11.9	10.6	29.1
71-98	0	0	18.1	40.9	8.0	12.5	20.5	42.0
98-120	0	0	41.3	34.0	6.5	7.0	11.2	24.7
120-140	0	0	37.2	37.8	5.4	6.6	13.0	25.0
140-160	0	0	24.7	39.8	6.3	11.2	18.0	35.5
160-200	0	0	45.9	30.1	5.2	9.5	9.3	24.0
Profile A5. Alluvisol humic with very strong deep stratified humic profile, loamy-clayey, moderately carbonate, arable, irrigated								
0-23	0	0	5.9	34.0	12.6	17.6	29.9	60.1
23-38	0	0	7.2	32.3	14.9	14.2	31.4	60.5
38-63	0	0	2.8	36.1	12.1	15.4	32.6	61.1
63-81	0	0	3.6	34.4	13.3	16.0	32.7	62.0
81-110	0	0	8.4	33.3	9.8	13.4	35.1	58.3
110-140	0	0	8.2	28.0	6.2	16.1	41.5	63.8
140-160	0	0	2.4	24.0	9.6	13.7	50.3	73.6
160-180	0	0	13.4	25.0	6.1	14.3	40.2	61.6
180-200	0	0	11.2	30.2	8.0	12.3	38.3	58.6
Profile A1. Alluvisol deep humiferous with a very strong humiferous profile, loamy, weak carbonate, arable, irrigated, post-marshy								
0-20	0	0	5.6	13.8	9.0	21.7	49.9	80.6
20-38	0	0	4.6	13.0	8.5	20.1	53.8	82.4
38-58	0	0	3.9	12.8	8.1	21.3	53.9	83.3
58-79	0	0	4.7	13.7	6.8	25.2	49.6	81.6
79-95	0	0	2.5	9.8	5.4	23.3	59.0	87.7
95-115	0	0	2.8	6.9	6.3	20.3	63.7	90.3
115-135	0	0	3.1	7.2	6.8	22.3	60.6	89.7
135-160	0	0	2.2	7.2	8.7	22.0	59.9	90.6
180-200	0	0	2.5	7.5	8.3	22.7	59.0	90.0
Profile 275. Alluvisol deep humiferous with a very strong deep humic profile, weakly carbonate, loamy, gleyed 62-130 cm, gleyic deeper than 130 cm, well-drained, post-marshy								
0-18	0	0.2	8.2	23.3	6.9	6.9	15.5	68.3
18-40	0	0.2	9.3	23.8	5.8	5.8	15.7	65.7
40-62	0	0.2	5.2	26.8	5.0	5.0	16.4	67.8
62-80	0	0.2	7.7	14.7	6.1	6.1	15.5	77.4
80-100	0	0.4	9.6	12.6	6.8	6.8	16.9	77.4
100-112	0	0.5	9.1	13.9	5.2	5.2	18.2	76.5
112-130	0	0.3	7.4	20.9	7.7	7.7	14.4	71.4
130-150	0	0.2	4.4	32.2	5.9	5.9	10.1	53.2
150-175	0	0	8.1	40.5	7.4	7.4	10.9	51.4
175-200	0	0	6.4	45.1	4.9	4.9	10.4	48.5

Genetic particularities and classification of the alluvisols in the Republic of Moldova.

According to the World Reference Base of Soil Resources (WRB 2014) the alluvial soil group *de facto* includes all soils formed on alluvial deposits, lacustrine deposits, alluvial deposits on dejection cones in river valleys. All these deposits and the soils spread over them are predominantly young stratified formations, formed during the overflowing of rivers. The stratified alluvial profiles are characterized by weak differentiation of pedogenetic horizons deeper than the horizon on the soil surface. The main qualifiers used to classify these soils are diagnostic characters (Soil map..., 1990; Cerbari, 2001; Егоров & Фридланд, 1977).

Hydric - it is used to classify alluvial soils from the point of view of the depth from the surface of the layer of soil saturated with water from the ground water account (Крупеников & Подымов, 1987). From the limit of the depth of the water-saturated soil layer, the following alluvium taxonomic units are divided:

- *hydric*, saturated in water starting from the land surface;
- *semihydric,* saturated in water in the depth range 35-70 (100) cm;
- *typical,* saturated in water deeper than 70 (100) cm.

Humic - it is used to classify alluvial soils from the point of view of humus content and the thickness of the humic profile. It is divided into the following subdivisions:

- *ochric* or poorly developed, the humus content in the surface layer of the alluvium in the Ao horizon is less than 1%;
- *poorly humiferous*, humus content 1-2% in the 0-30 cm layer or the arable layer;
- *humic,* alluvial soils with a weighted average content of humus in the 0-50 cm layer greater than 2%.

Deeply humic - is used to name alluvial soils with a weighted average content of humus greater than 2.5% in the 100 cm layer.

Gleyed - it is used to divide alluvial units according to the degree and depth of glazing;

Gleyic - is used to divide alluvial units according to the depth of the gleyic horizon (horizon with a heavily gleyic surface (gleyic) greater than 90%).

Salic (salinized) - is used to divide soil taxonomic units according to the degree and depth of the appearance of salinization or solonetization on the soil profile etc. (Table 2).

Table 2. Systematic list of alluvial soils of the Republic of Moldova at subtype level

Upper level of soil taxon: ALLUVISOLS	
Lower level of soil taxon:	
- ochric (poorly developed)	- the humus content in the 0-20 cm layer is less than 1%.
- typical	- weakly humiferous, humus content in the 0-30 cm layer is 1-2%.
- humic	- weighted average humus content greater than 2% in the 0-50 cm layer.
- deeply humic	- weighted average humus content greater than 2% in the 0-100 cm layer.
- slitizated	- the apparent density of the unworked subarable layer is >1.6 g/cm^3.
- hydric	- marshy, the 0-30 cm soil layer is saturated in water.
- semihydric	- semi-marshy, the 30-70 cm soil layer is saturated in water.
- salinized	- it is divided according to the depth and degree of salinization of the salinized soil layer.
- solonetizated	- it is divided according to the depth and degree of solonetization of the solonetized soil layer.
- gleyed	- it is divided according to the depth and degree of gleizated of the glazed soil layer.
- gleyic	- it is divided according to the depth of the location of the gleyic layer.

Ochric alluvial soils are defined by a weakly evolved Ao horizon no more than 20 cm thick with a humus content of less than 1%, followed by parent material consisting of alluvial (fluvial), alluvial-lacustrine or recent lacustrine deposits of any texture. These soils are formed in conditions of more or less regular overflows of running water. In the intervals between overflows, it is possible to manifest solification, the intensity of which is all the greater, the longer the time since the last overflow (Ursu, 2011; Парникова, 2020).

In the meadows or parts of the meadows, out of the influence of overflows or flooded only at long time intervals, solification advances leading to the transformation of poorly evolved alluvial soils (ochric) into typical alluvial soils. So, the alluvial soils, as a result of the extremely different conditions regarding the duration of the manifestation of solification, the climate of the area, the origin of the alluvial deposits, their texture and composition, the depth and mineralization of the groundwater, are characterized by a very large variation in the properties and composition of their profile.

Poorly developed alluvisols have a profile of the type: AC - 1C - 2C - 3Cg. They are spread in the meadows of the Prut and Dniester on young relief units (minor beds), which carry out intense actions of transporting and depositing alluvial material.

Alluvisols formed on homogeneous deposits have a uniform texture, and those formed on inhomogeneous parent material have a contrasting texture. As a rule, poorly developed alluvial soils are unstructured. The accumulation of organic matter in these soils is poorly expressed. It is characterized by a low content of humus around 0.5-1.0%, coming from the respective deposits or formed due to the organic matter resulting from the weak vegetation spread on these soils (Didenco, 2022; Leah & Cerbari, 2019).

Typical alluvisols are formed on unflooded high meadows or on parts of meadows that are flooded at longer time intervals (Думих, 2016). In such situations, the development of vegetation, the manifestation of solification in the formation of a moderately evolved humiferous horizon was possible. They have a profile of the type: Ah - 1Ah - 2Ah - 1Cg - 2Cg. The thickness of the humiferous profile is 40-50 cm and more. The texture of the profile, depending on the homogeneity of the alluvial deposits, can be uniform or contrasting. Typical alluvisols have a glomerular, granular or polyhedral structure, poorly to moderately developed. In comparison with the poorly evolved alluvisols, they have a higher humus content than the typical ones - 1-2% (Leah et al., 2019).

The ***hydric*** and ***semi-hydric*** alluvisols are spread in the recently abandoned riverbeds and were formed due to silty alluvial deposits in the long-term presence of groundwater at a depth of 35-70 cm from the surface. They have the profile of the type: Ahp - 1Ahg - 2ACr - Gr or ACg - ACr - Gr. The humiferous profile is weakly or moderately developed, and the gleization process is strongly expressed. Humus content is 1-3% or even higher.

Humic and ***deeply humic*** alluvial soils are mainly spread in the lower meadow of the large Dniester and Prut rivers and in the lower meadow of small rivers, at their discharge into the Dniester and Prut rivers. The formation mechanism of these soils is further described for the meadow of Lower Botna and Lower Dniester (Leah, 2018; Didenco, 2022).

At the moment, the territory of the Lower Dniester meadow is used for arable land and is characterized by an unstable favorable water table regime. In case of irrational soil and water management, the unstable favorable hydrological regime can become unstable unfavorable (which happens as a result of incorrect irrigation).

The arable deep-humic post-swampy arable alluvisols of the Lower Dniester meadow are weakly carbonated and are characterized by favorable pH values in the range of 7.2-7.4. An extremely important index of these soils is the very high content of mobile phosphorus throughout the humiferous profile, up to a depth of about 2 m (>10 mg/100 g soil).

Such soils, with analogous historical conditions of solification - gradual synergetic accumulation over thousands of years of fine alluvial - proluvial deposits mixed with organic ones, rich in mobile phosphorus, formed in conditions of marsh pedogenesis, are very rare. Phosphorus is a strategic element that makes possible the use in ecological agriculture of the deep-humic post-marshy alluvial soils drained from the Lower Dniester meadow.

CONCLUSIONS

The process of pedogenesis in synergistic conditions of the marsh and the periodic overflow of the Lower Dniester over the course of millennia, then the desiccation and the use of arable land for about 60 years led to the formation today in the Lower Dniester meadow of post-marshy deep-humic alluvisols (the humus content in the upper layer is 4-5%) with extremely deep humiferous profile with a depth up to 1.7-2.0 m, very rich in mobile phosphorus. At the moment, the soil cover of the Lower Dniester meadow is used in non-irrigated agriculture. In the post-Soviet period, about 30 years ago, after the drying up of the 60s, the lands with these endemic soils were irrigated;

after the land reform, the irrigation system was completely damaged.

The investigated soils are characterized by a high level of natural fertility, they are rich in humus and mobile phosphorus, it is only necessary to restore the unfavorable physical condition of their arable layer by increasing the flow of qualitative organic matter in this layer.

Reducing the negative consequences of climate change that directly influences the degradation of the previously arable layer 0-30 or 0-35 cm is possible only by switching to green agriculture that provides for the systemic use of the green mass of leguminous ameliorating plants (peas or autumn and spring peas) as an organic fertilizer in couple with the gradual periodic implementation of the Mini-till tillage system.

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REVIEW OF *EX SITU* RESEARCH METHODS REGARDING THE PLANT - SOIL FAUNA RELATIONSHIP

Luiza Silvia CHIRIAC[1, 2], Dumitru T. MURARIU[1, 2]

[1]Institute of Biology Bucharest, Romanian Academy, 296 Splaiul Independentei, Bucharest, Romania
[2]Faculty of Biology, University of Bucharest, 91-95 Splaiul Independentei, Bucharest, Romania

Corresponding author email: luizaschiriac@gmail.com

Abstract

While the plant community controls the quality and quantity of resources available to soil invertebrates, the soil invertebrates regulate plant growth and plant community composition. Soil invertebrates can modify plant traits, this effect cascading up to higher trophic levels, potentially thus determining changes in ecosystem functions. Thus, considering the special importance of this relationship between plants and soil invertebrates, our work aims to identify the various methods that support the study of this relationship. Although following the critical analysis of the literature, multiple methods were identified that highlight the interactions between underground and aboveground communities, we cannot claim that the study is exhaustive, which is caused by the immense number of works in the field. This aspect can only pave the way for new works and experiments to fill the knowledge gaps in this thematic area.

Key words: *ex situ methods, methods, plants, plants-soil fauna relationships, soil invertebrates.*

INTRODUCTION

The bidirectional relationship between invertebrate and plant groups is used as a bioindicator, providing information about soil quality, ecosystem services and ecosystem functioning (Scheu, 2002; Manu et al., 2019). Within different types of ecosystems there is a great diversity of functional traits of plants and soil invertebrates. Soil invertebrates play a key role in maintaining soil health and sustainability. Many species of invertebrates influence soil fertility and are very important in crop production and productivity (Manu and Onete, 2013). Soil fauna also is important in the regulation of nutrient cycling, in the decomposition process and also they can function as buffer organisms for various types of impacts (Carillo et al., 2011). The phylogenetic relationship between various invertebrate species is an indicator of abilities or constraints, on their morphology, physiology, and behavior. The question that arises is whether this relationship represents a sufficient condition for predicting ecosystem services and system behavior in the future (Walter and Ikonen, 1989). Ecological interactions occur between individuals and can change with life stage, season, time of day, physiological need, or in response to many other variables (changes within the food web, pollution, or other types of anthropogenic impact). The conceptual approach to plant-invertebrate interactions uses plant functional traits and soil food web characteristics (Cifuentes-Croquevielle et al., 2020). An important step in this approach is to include energy flow between species in the food web (e.g. building an energy flow network) (Scheu, 2002). Since plants use nutrients in inorganic form, they depend on the rate of mineralization in the soil (Araujo et al., 2004). Nutrient mineralization is mainly the result of the activity of soil fauna. The soil benefits from a great diversity of plant and invertebrate species, from a dense trophic network where the species living in it depend on its quality. Climate and land use changes cause modifications in all ecological systems including the structure and distribution of invertebrate communities (Wurst et al., 2018). Some studies mention that the soil cannot self-regulate its characteristics when links in the trophic network are impacted by rising temperatures, loss of moisture, pollution, etc. Due to the changes in the dynamics of the invertebrate community, there are also changes in the functional traits of the plants (Robinson et al., 2018). Soil fertility represents its ability to provide nutrients necessary for the growth and development of plants (Culliney, 2013) taking into account their ecological requirements (Boháč, 1990). A fertile soil can be defined either based on its properties or based on plant production and productivity (Benton Jones, 2012). Fertility is determined by physical factors (texture, structure, profile depth, water retention capacity, drainage capacity), chemical (pH, quantity of essential elements available to plants, ion exchange capacity, organic and mineral matter content) and biological (soil organisms, dominance-abundance ratio, inter- and intraspecific relationships) (Chiriac et al., 2020). Crop productivity and soil fertility can be affected due to the lack of essential elements. Sustainable soil management not only aims to improve crop productivity but also soil fertility and sustainability (Khalid et al., 2019). Studying all the species plus all their interactions is a daunting task, even in a simple ecosystem. In more complex systems it is impossible. Therefore, researchers need to reduce this complexity to a manageable size. In this sense, the aim of this literature review was to identify methods used by various authors to study these relationships outside their area of origin, in laboratory.

MATERIALS AND METHODS

The methods used to carry out this literature review included the following stages: public databases were queried (Web of Science, Google Scholar, Scopus), then the critical analysis of the specialized literature was carried out. All this information was compiled into a comprehensive Excel database containing many details from the literature reviewed. To carry out the query stage we used the keywords such as: microcosm, plant-invertebrate relationship, vegetation chamber, ex-situ methods for researching this relationship, etc. The articles used were exclusively free access or articles that were provided to us directly by the authors following the request made on certain platforms (ResearchGate). From all the articles studied, only those of them that contained the information targeted in this study were included in the present paper.

RESULTS AND DISCUSSIONS

Many ecological hypotheses cannot be proven with field studies, so experimentation in laboratory studies is used to provide insights into otherwise inaccessible interactions and mechanisms. The growing interest in recent years in the relationship between aboveground processes and soil ecology has been facilitated by the widespread use of laboratory experiments to overcome the limitations of understanding imposed by the particularly complex nature of the subsurface environment (A'Bear et al., 2014). Climate simulation chambers (growth chamber, vegetation chamber or phytotron) offer the possibility to test concepts in ecology and evolution, using different groups of organisms, including bacteria, algae, arthropods, etc. (Altermatt et al., 2015). These are artificial, simplified systems used to simulate the behavior of natural ecosystems under controlled conditions. They have long been used in ecology to increase scientific understanding of natural processes (A'Bear et al., 2014). In the context of changes in environmental conditions, the interactions of species on the surface of the soil and those in the subsurface represent a major concern in recent years. An important driver of these processes is the feedback between plants and soil invertebrate communities (Chiriac et al., 2020). Also, this type of experiment allows direct estimation of the effect of climate change on certain demographic traits (fertility, mortality, population growth rate, population density, etc.). These estimates can be used in population models to determine the extinction risk of a population in the absence of immigration or emigration (Cao et al., 2021). Experiments using a simulated climate chamber have the main advantage that they allow most variables to be held constant while only a few are manipulated, thus providing detailed insights into the ecology of soil plant-invertebrate interactions (A'Bear et al., 2014).

The mesocosm is defined in the literature as an experimental enclosure with a capacity of one liter to several thousand liters, for highlighting and clarifying the processes that occur during climate change (Stewart et al., 2013). Most subject areas in biology use certain models to explain phenomena. In the case of ecology, there are not a large number of models because everything depends on many different variables from year to year. The vegetation chamber is worth considering to create such models and make various predictions. Models need to have three useful characteristics: tractability, generality, and realism, which allow future experiments to build on past results (Srivastava et al., 2004). Greenhouse experiments were long ago considered irrelevant because it was believed that they could not be replicated and that there was no randomization between experimental designs (Lee and Rawlings, 1982). It is interesting, however, how a single term can have different meanings depending on the purpose and objectives of each study (Table 1). Also, different authors used a very different number of experimental variants. This is caused by the purpose and objectives of each individual work, the scale at which a certain experiment is carried out (Constantinescu et al., 2019), the variables included (amendment, inoculum, different types of soil) or the number of species of plants under study (a single species or a combination of several species). The number of pots/containers/pots of vegetation must be chosen very carefully, so that the requirements of performing some statistical methods are met, but also to capture the trends pursued by the study.

Table 1. Types of containers used in laboratory experiments by various authors over time

Year	**Authors**	**Containers**
1998	Fraser and Grime	35 outdoor, closed, ventilated plastic containers
1998	Salamanca et al.	plastic tubes in the growing chamber
1999	Scheu et al.	56 plastic tubes in the growing chamber
2000	Buckland and Grime	72 outdoor, closed, ventilated plastic containers
2001	Bonkowski et al.	48 plastic tubes
2003	De Deyn et al.	32 plastic containers in the growing chamber
2004	Cole et al.,	190 vegetation pots in the greenhouse
2009	Nygaard and Ejrnæs	72 outdoor, closed, ventilated plastic containers
2009	Aira and Piearce	30 vegetation pots outdoors
2010	Hedde et al.	glass jars with lids in greenhouses
2012	Borchard et al.	16 vegetation vessels in vegetation chambers
2012	Păun et al.	12 vegetation pots in vegetation chambers
2013a	Neagoe et al.	70 vegetation pots in the vegetation room
2013b	Neagoe et al.	48 vegetation pots in the vegetation room
2014	Nicoară et al.	10 vegetation pots in vegetation chambers
2015	Yang et al.	60 vegetation pots in vegetation chambers
2017	Panteleit et al.	100 plastic boxes with transparent lid
2019	Constantinescu et al.	5 vegetation pots in vegetation rooms
2020	Lebrun et al.	70 pots in the growing room
2021	Rubio-Ríos et al.	120 vegetation pots in vegetation chambers
2022	Balacco et al.	36 vegetation pots in the vegetation chamber

Vegetation chambers have been used since a decade and a half ago, being the most feasible for carrying out complex experiments accurately.

The authors of the analyzed studies used very different variables (temperatures, humidity and circadian cycles) (Table 2). This is due to the distinct purpose of each individual item. For example, Rubio-Rios et al. (2021) in their experiments used a temperature of 10°C because this was the average value of hourly records obtained during the same period of the experiment in previous years and a light/dark photoperiod of 12:12 h based on the length of the natural day cycle in that time of year. Usually, the variables to be set are based on knowledge of the ecological requirements (optimal values) of the species used in the experiment. One such study adapted the chosen day/night cycle in the vegetation chamber according to the species chosen in the experiment. Although initially the light period was set to 14 hours and the dark period to 10 hours (during the seeding period of the plant species chosen in the experiment), when the snail species was introduced into the experiment, the hours were changed as follows: 18 hours of light and 6 hours of darkness because this was more suitable for the growth of snails. In both periods, the temperature remained constant (23°C/17°C) (Scheifler et al., 2006). In other situations, the temperatures chosen are several degrees higher than the optimum of those species because the purpose of these studies is to make predictions of the behavior of various plant species in the current context of climate changes.

Table 2. Environmental variables used by various authors in greenhouse experiments over time

Year	**Authors**	**Temperature (degrees day/degrees night)**	**Humidity**	**Circadian cycle (hours day/hours night)**
1982	Lee et al	26°C/22°C		
1999	Scheu et al.	18°C/18°C		
2001	Bonkowski et al.	20°C/15°C		16 h/8 h
2003	De Deyn et al.	21°C/16°C		16 h/8 h
2004	Cole et al.			12 h/12h
2005	Bezemer et al.	20°C/14°C	60%	16 h/8 h
2006	Scheifler et al.	23°C/17°C		18 h/6h
2009	Aira and Piearce	20°C/20°C		
2010	Hedde et al.	10+/-1°C		11 h/13h
2013	Păun et al.	16°C/22°C	60%	
2013a	Neagoe et al.	22°C/16°C	60%	16 h/8 h
2013b	Neagoe et al.	22°C/16°C	70%	16 h/8 h
2014	Nicoară et al.	25-35°C /15-25°C		
2017	Panteleit et al.	27°C/27°C	90%	12 h/12 h
2017	Neagoe et al.	22°C/16°C	70%	16 h/8 h
2020	Lebrun et al.	24° C/21° C		16 h/8 h
2021	Rubio-Ríos et al.	10°C/ 10°C		12 h/12 h
2022	Balacco et al.	24°C/16°C	50%	12 h/12h

In the laboratory studies, the authors used plant species (Table 3) as indicators of certain types of changes (climate, land use, etc.) (Blouin et al., 2013). One of the studies manipulated plant functional diversity (monocultures and mixtures of low functional diversity and high functional diversity) in the presence and absence of detritivores and assessed the effects on litter decomposition, nutrient cycling and fungal and detritivore biomass. This study obtained positive effects of diversity on decomposition by detritivores. Among the species very often used in pot experiments introduced into the vegetation room is *Agrostis capillaris*. In one of the pots, the effects of microarthropod species diversity (Collembola) on nitrogen distribution between *Agrostis capillaris* and soil microbial biomass were tested to determine how the richness and diversity of soil fauna influences plant-microorganism competition for organic nitrogen (Cole et al., 2004). Another experiment using *Agrostis capillaris* alongside *Anthoxanthum odoratum* aimed to manipulate the composition of the belowground (no soil inoculation, invertebrate inoculation, microorganism inoculation or both invertebrate and microorganism inoculation) and aboveground (aphids and parasitoids) community composition to measure individual performance and population dynamics of introduced species. The authors were able to demonstrate that aboveground multitrophic interactions are influenced by the composition of belowground communities and thus, aboveground plant–insect links cannot be viewed independently of rhizosphere interactions (Bezemer et al., 2005).

Table 3. Plant species used in laboratory experiments by different authors

Species	**Nr. of articles**	**Article**
Achillea millefolium	1	Bezemer et al., 2005
Agrostis capillaris	6	De Deyn et al., 2003; Bezemer et al., 2005; Neagoe et al., 2013a; Wernitznig et al., 2013; Nicoară et al., 2014; Constantinescu et al., 2019
Anthoxanthum odoratum	2	De Deyn et al., 2003; Bezemer et al., 2005
Arrhenatherum elatius	1	Fraser și Grime, 1998
Campanula rotundifolia	2	De Deyn et al., 2003; Bezemer et al., 2005
Capsella bursa-pastoris	2	Johnson et al., 2011; Wagg et al., 2014
Cerastrium fontana	1	Bezemer et al., 2005
Deschampsia flexuosa	1	Wernitznig et al., 2013
Festuca ovina	3	Fraser și Grime, 1998; De Deyn et al., 2003; Bezemer et al., 2005
Festuca rubra	3	De Deyn et al., 2003; Wernitznig et al., 2013; Nicoară et al., 2014
Helianthus annuus	1	Păun et al., 2012
Hordeum vulgare	1	Johnson et al., 2010
Lactuca sativa	1	Scheifler et al., 2006
Lolium multiflorum	2	Borchard et al., 2012; Wagg et al., 2014
Lolium perenne	1	De Deyn et al., 2003; Balacco et al., 2022
Lotus corniculatus	2	Bezemer et al., 2005; Wagg et al., 2014
Lupinus angustifolius	2	Neagoe et al., 2005; Vișan et al., 2007
Lycopersicon esculentum	1	Yang et al., 2015
Nicotiana tabaccum	1	Neagoe et al., 2017
Oryza sativa	1	Panteleit et al., 2017
Phacelia tanacetifolia	1	Neagoe et al., 2013b
Plantago lanceolata	3	De Deyn et al., 2003; Bezemer et al., 2005; Wagg et al., 2014
Poa annua	3	Fraser și Grime, 1998; Scheu et al., 1999; Wagg et al., 2014
Poa trivialis	1	De Deyn et al., 2003
Prunella vulgaris	3	De Deyn et al., 2003; Bezemer et al., 2005; Wagg et al., 2014
Rumex acetocella	1	Bezemer et al., 2005
Rumex acetosa	1	Scherber et al., 2006
Rumex obtusifolius	1	De Deyn et al., 2003
Secale cereale	3	Neagoe et al., 2005; Vișan et al., 2007; Păun et al., 201
Senecio jacobaea	1	Bezemer et al., 2005
Senecio vulgaris	1	Johnson et al., 2010
Sinapis alba	1	Neagoe et al., 2013b
Stellaria media	1	De Deyn et al., 2003
Trifolium pratense	3	Scherber et al., 2006; Neagoe et al., 2013b; Wagg et al., 2014
Trifolium repens	1	Scheu et al., 1999
Tripleurospermum	1	Bezemer et al., 2005
Triticum aestivum	1	Bonkowski et al., 2001
Glycine max	1	Lee și Rawlings, 1982
Legumes, grasses, forbs	8	Vagg et al., 2014
Grasses and forbs	1	Buckland și Grime, 2000
grasses, forbs, and two woody species, *Echinacea purpurea*	1	Dybzinski et al., 2008
Deciduous, semi-deciduous, evergreen species *A. glutinosa* as key species	1	Rubio-Rios et al., 2021
grasses, legumes, small herbs, tall herbs	1	Partsch et al., 2006

Invertebrates were also widely used in laboratory experiments (Table 4). Earthworms, for example, have been recognized as ecosystem "engineers" and represent an excellent potential partner for humans in managing ecosystem services (Blouin et al., 2013). One such study investigated the effect of collembola and earthworms on *Poa annua* and *Trifolium repens* species.

Table 4. Invertebrate species used in the laboratory by different authors

Invertebrate species used in the laboratory	Nr of articles	Article
aphids	4	Fraser și Grime, 1998; Bezemer et al., 2005; Buckland and Grime, 2000; Johnson et al., 2010
annelids	1	Panteleit et al., 2017
mycorrhizal arbuscular fungi	4	Păun et al., 2012a, Neagoe et al., 2013 a, b; Neagoe et al., 2017; Balacco et al., 2022
arthropods	1	Panteleit et al., 2017
springtails	5	Scheu et al., 1999; Deyn et al. 2003; Cole et al. 2004; Partsch et al. 2006; A'Bear et al., 2014
diplopoda	1	A'Bear et al., 2014
earthworms	5	Scheu et al., 1999; Bonkowski et al., 2001; Partsch et al. 2006; Johnson et al. 2010; Hedde et al., 2010
herbivorous	1	Buckland and Grime, 2000
ground beetle	1	Buckland and Grime, 2000
isopoda	1	A'Bear et al., 2014
ladybird	2	Fraser și Grime, 1998; Buckland and Grime, 2000
trichopters	1	Rubio-Ríos et al., 2021
microorganisms	4	Bezemer et al., 2005; Păun et al., 2012b; Wernitznig et al., 2013; Nicoară et al., 2014
millipedes	1	Hedde et al., 2010
mites	1	Cole et al., 2004;
nematodes	3	Deyn et al., 2003; Bezemer et al., 2005; A'Bear et al., 2014
parasitoids	2	Bezemer et al., 2005; Johnson et al., 2010;
protozoa	1	Bonkowski et al., 2001
snails	2	Scheifler et al., 2006; Panteleit et al., 2017
oniscoides	1	Hedde et al., 2010

The authors started from the hypothesis that the soil used has a low amount of nitrogen available for plants and that the invertebrate species used could increase this amount. Earthworms caused a more than two-fold increase in the biomass of the plant species studied (Scheu et al., 1999). This is also confirmed by another study that demonstrated that decomposers (earthworms and collembola) influence soil structure and nutrient mineralization, as well as the activity and composition of the soil microbial community and therefore affect plant production and productivity (Partsch et al., 2006). The effect of earthworms on seed germination of *Lolium perenne* and *Agrostis capillaris* was also studied. The seeds of these species were added to vegetation pots at different depths, some with earthworms, others without, the experimental variants being improved with compost based on fungi (expanded clay). Germination was examined as a function of seeding depth and viability of seeds passed through the digestive tract of earthworms. Earthworms drastically reduced the germination of *Agrostis capillaris* seeds, but did not affect the germination of *Lolium perenne* seeds (Aira and Piearce, 2009). This was probably caused by the larger seed size of *Lolium perenne*. A similar experiment aimed to test the influence of earthworms and protozoa on phytophagous aphids on a host plant (*Triticum aestivum*). Both groups of animals significantly increased plant development, but the effects of protozoa exceeded those of earthworms at least twice, and aphids were more strongly influenced by protozoa than earthworms (protozoa caused increases in the number of juveniles and adults of aphids on each plant). The experimental variants also included microcosms with only protozoa or only earthworms, the ideal variant being when they were added together, being found to have a cumulative effect (Bonkowsky et al., 2001). Also in the laboratory, studies were carried out that used snail species. Both papers used the microcosm, but in the first article an evaluation of the transfer of heavy metals in the soil-plant-snail trophic chain was pursued, and the second aimed at quantifying the influence of detritivores (including snails) on straw decomposition of rice under flood conditions (Scheifler et al., 2006; Panteleit et al., 2017).

CONCLUSIONS

From a chronological point of view, I observed that the methods initially described were generally interested in the response of plants and/or invertebrates to certain changes in the environment. Currently, the studies are much more complex, they are multivariate, the researchers trying to find out the answer of the community chosen in the study in as complex a framework as possible. With the development of knowledge and the accumulation of experience of researchers, the studies were carried out at a more specialized level, scientists trying to conduct them in the laboratory. In recent years, the trend is towards studies carried out in vegetation chambers, where researchers could modify the environmental variables (temperature, humidity, circadian cycle) and the species used (plants, invertebrates, microorganisms) so that their results are as accurate in terms of forecasting the effects of global changes on the plant and invertebrate species studied. Over the past 30 years, more and more studies have appeared that aim to understand surface-subsurface interactions. The literature includes many papers that highlight the fact that these interactions have an essential role in the functioning of terrestrial ecosystems and in the provision of much-needed ecosystem services.

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EXPLOITATION OF AGRICULTURAL LAND - RESULT OF THE INTENSIFICATION OF LAND SUBJECT TO DEGRADATION IN THE NISPORENI DISTRICT

Olesea COJOCARU, Alexandru VERBIȚCHI

Technical University of Moldova, 50 Mircesti Street, Chisinau, Republic of Moldova

Corresponding author email: olesea.cojocaru@am.utm.md

Abstract

In recent decades, the intensification of multiple forms of soil degradation has been recorded, especially that through erosion due to inappropriate intensive exploitations. The purpose of the research is to examine the condition of the agricultural lands in the Nisporeni district and what is the weight of their exploitation, by proposing measures to remedy them. Nisporeni district is located in the central part of Moldova, in the wooded area. Nisporeni district is a component of the „Siret-Prut-Nistru" Euroregion. The geographical position favors access, communications and trade of the district at the border with the European Union. In the structure of agricultural holdings, the lowest share of multi-year plantations is in Frontier and in the North-West. Over 50% of the district`s land fund is concentrated in the Nisporeni and Nord-Est microzones, which also own over 46% of agricultural land and approx. 60% of the forest fund. The cost of washed soil is about 1.85 billion lei, and the cost of agricultural production losses - about 0.873 billion lei. Thus, the direct and indirect damage caused by erosion is 2.723 billion lei.

***Key words**: agricultural lands, exploitation, degradation, Nisporeni district.*

INTRODUCTION

Land resources are considered not only as a territorial and natural basis, but also from the point of view of economic content, as an object of ownership and management: land is the basis of agricultural production, a type of real estate and a spatial basis for the construction of buildings, structures, infrastructure. Without the participation of land in the production process, it is impossible to obtain material benefits, which is a unique quality of nature. It is the spatial limitation of the land that is its specific feature as a production resource. The earth cannot be reproduced or artificially increased its resources. And finally, land resources are the main component of natural resources, and land use, respectively, is the main type of nature management. The purpose of land management is to provide the population with a high level of economic, social and environmental living conditions, taking into account the conservation and restoration of natural resources. Due to the fact that any human activity is inextricably linked with the earth, it can be concluded that the earth is a fundamental element for any human activity (Cerbari et al., 2010; Ursu, 2000; Антропов et al., 2018; Васильева, 2018; Шубич et al., 2019).

Any human activity is regulated by his rights. Land plots, in turn, are objects of civil rights. Land is a reliable investment object, a basic element of property relations and the main component of the real estate market. For this reason, land protection and rational land use are an essential condition for the development of the state and the growth of the welfare of its citizens (Сидоров, 1965; Татаринцев, 2011).

Rational land use is ensured as a result of the organization of land use management. In the economic literature, there are many definitions of management (management), which focus on its individual features, a special type of activity, the process of creating stimulating production conditions, impact, etc. (Комова, 2020).

Effective land management makes it possible to develop entrepreneurial and social activities, form an economically sound taxation system with subsequent collection of budget revenues, attract investments in the development of municipalities and regions, and create an effective system for ensuring rights and guarantees for subjects of land relations. Therefore, when analyzing land use, it is necessary to take into account the socio-economic situation in the country, national characteristics, as well as historical traditions and the mentality of citizens (Антропов et al., 2018).

The depletion of land resources due to their scarcity leads to the identification of problems related to land ownership and taxation of land resources. Therefore, the main element of the system of state economic regulation of land use is the valuation of the land plot and land payments determined on the basis of its results, since the land management mechanism is based on payment, an important element of economic regulation of land use (Будина, 2013).

In the 19th century, the acceleration of urbanization led to an increase in the concentration of the population in cities, which became possible due to the development of industry, transport and communications, the intensification of agricultural production, the improvement of knowledge in the field of medicine, etc. The share of the urban population in the world for the period from 1800 to 1990 increased from 5.1 to 41.3% of the total population (Ursu, 2006; Дмитриев et al., 2018).

It should be noted that human life processes have negative environmental consequences, which, for the most part, affect the state of land resources (Меньших et al., 2016).

Anthropogenic activities are mainly associated with the land, which is irrefutable proof of the paramount importance of this natural resource. In particular, the ecological component of land use is also important; therefore, special attention has been paid to the ecology of cities in recent years. The development of urbanized territories is accompanied by the development of land plots, the change (technical re-equipment) of buildings, structures and other real estate objects. So, the trend that is relevant today, which appeared in the last century, involves the acquisition of land plots with obsolete enterprises or production stopped on them, and then the demolition of old buildings and the construction of new investment-attractive real estate objects. This process of real estate transformation is called redevelopment and is one of the methods of managing urban land use of industrial areas (Andrieș, 2011).

The founder of genetic pedology, V. Dokuceaev, about 120 years ago found that the soils of Moldova contained from 5 to 9% of humus. Humus reserves in the 0-30 cm layer constituted about 200 t/ha. During 50-70 years of extensive exploitation of agricultural lands, without soil fertilization, the humus content decreased by 1-3% and at the beginning of the 50s it was 4-5%. Towards the period of intensive chemistry (1965-1970) the amount of organic matter was reduced to 3.5-4.0%. In a period of 90 years, humus reserves decreased by 70 t/ha, nitrogen by about 3.5 t/ha. It is predicted that the amount of humus will decrease considerably by 2025 and will constitute only 2.5-3.0%, and the total nitrogen reserves - 4 t/ha. Currently, about 41% of the total area of agricultural land is characterized by a low content (less than 2%) of humus, 40% moderate (2-3%) and only 20% with a relatively high content (more than 3%). About 80% of soils have a very low and low nitrification capacity. The situation is identical with the 80% of soils, which contain less than 3.0% of humus. Of the nutrients, the first minimum is nitrogen and phosphorus. The surface of soils with low content of mobile phosphorus constitutes 31%, moderate 34% and only 35% high. In the last 10-12 years, the volume of mineral fertilizers incorporated into the soil has decreased by 15-20 times, organic fertilizers by 25-30 times. The balance of humus and nutrients is profoundly negative. Soil fertility gradually decreases, as a result the phenomenon of desertification, biological and chemical degradation intensifies (Andrieș, 2011; Bejan, 2010; Cerbari et al., 2010; Ursu, 2000).

Nature management is the practice of human use of the natural environment and natural resources, which is a system of relationships between man and nature. The most important element of nature management is land resources - this is the surface of the earth on which a person conducts economic activities: lives, builds, cultivates (Васильева, 2018; Шубич et al., 2019).

Anthropogenic activity causes damage to the soil layer, which leads to insufficient restoration of soil formation functions and a negative change in its structure. Over time, soil properties tend to deteriorate, change their chemical composition, become polluted, degrade and, in some cases, become unusable. It is worth noting that the basis of land use management is the rational use of natural resources, the prevention of a decrease in its productivity and quality (Ursu, 2000).

Every year in the spring, work is carried out on the agricultural lands for the sowing of agricultural crops. Farmers prepare seeds and soil for sowing. These works are characteristic of any farming system and no one at this time is interested in what processes or products in the field in the period after the harvest and until the beginning of the spring works. It is obvious that knowledge about the rational use and conservation of soil moisture is necessary for all economic agents in agriculture. For these reasons, knowledge regarding the rational use of soil water needs to be provided by scientific research in this direction (Kuharuk et al., 2015).

MATERIALS AND METHODS

The research was carried out in the Nisporeni district, which includes 23 town halls, within which there are 39 localities (Figure 1). The seat of the district is the town of Nisporeni. The main materials used: normative legislative acts related to the research object; Regional Development Strategies for the period: 2020-2022; Statistical yearbooks on the quality of environmental factors. The annual reports of the Ecological Agencies and Inspections. Main methods used: administrative sources; statistical data, bibliographic sources, comparative analyses of assessment of climatic conditions, assessment of soil condition under the influence of anthropogenic factors.

The district comprises 2.4% of the total area of the Republic of Moldova (62.9 thousand ha), being adjacent to the North - with Ungheni and Călăraşi districts; to the South - with Hânceşti district; to the East - with Străşeni district; in the West - with Ungheni district and Iasi county in Romania. Nisporeni district is a component of the "Siret-Prut-Nistru" Euroregion. The geographical position favors access, communications and trade of the district at the border with the European Union. The district is crossed by the transport route - international car: M 1 - Chişinău-Leuşeni. The total area of the district is 62.9 thousand ha, of which: arable land - 21.6 thousand ha, perennial plantations - 8.6 thousand ha, the lands of the state forestry fund - 15.4 thousand ha; the lands of the state water fund - 1.6 thousand ha.

Nisporeni district is located in the central part of Moldova, in the wooded area. To the North it borders Călăraşi district, to the South with Hînceşti district, to the West with Ungheni and Romania districts, to the East with Străşeni district.

Figure 1. Administrative-territorial representation of Nisporeni district

RESULTS AND DISCUSSIONS

The physical-geographical position of the Nisporeni district is advantageous, the relief, the mild temperate continental climate, the fertile chernozem soils favored the population and exploitation of this territory. The relief is hilly and fragmented by valleys, ravines, which occupies most of the arable land. Near the village of Bălănești is the highest hill in the country with an altitude of 429.50 m and which bears the same name as the village - Bălănești.

The climate is moderate-continental, characterized by an unstable character. The average air temperature is equal to 8-9°C, and at the surface of the soil between 10 and 11°C. The sum of positive temperatures is maintained for 9 months. The average temperature of January in the region is -4°C. The absolute minimum of temperatures is 33^{0}C, and the maximum is + 40°C. The cold periods of the year are short. The first frosts appear in October, the last in April. The duration of the frost-free temperature period is 174-179 days.

The largest annual amounts of precipitation are 500-550 mm, which fall on the western slopes of the central Moldavian plateau. The annual amount of precipitation in Nisporeni is 479 mm. The largest amount of precipitation (55-85%) falls during the vegetation period (April - November). Solid precipitation is especially characteristic for the months of January - February. The first snow falls at the end of October, in December the snow cover becomes stable. In the central part of the country, including Nisporeni, the snow lasts 60 days. The Nisporeni district is one of the regions of the country with an increased risk of hail. The highest number of days with hail per year varies from 4 to 8. Nisporeni district has rich agroclimatic resources, which favors the development of all cereal and vegetable crops; vines and fruit trees.

The main branches of the economy of the Nisporeni district are (Figure 2): agriculture and the processing industry of agricultural production, services and trade.

The degree of diversification of the economy is extremely low, especially through small trade and service enterprises.

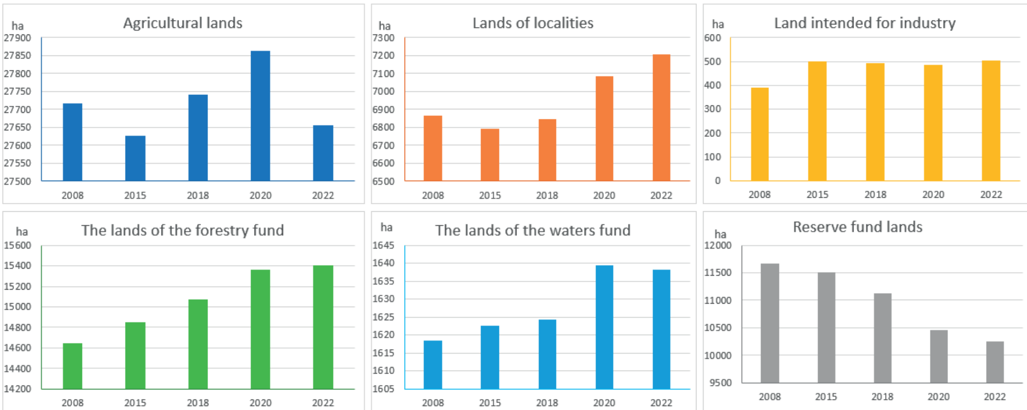

Figure 2. Representation of the land use gap from 2008-2022 on the territory of the Nisporeni district

The Nisporeni district is predominantly agricultural based on small individual agricultural owners and a limited number of enterprises that process local production up to the finished product. SMEs recently have a sustained growth trend (approx. 18%), and the number of peasant households has decreased by over 20% due to reorientation towards other types of activity or due to migration. The basic activity in the economy of the district is agriculture, but in most cases the entrepreneurs and peasant households practicing agriculture do not respect the ecological and agrotechnical rules, most of the agricultural households are located on steep slopes (up to 25 - 30 degrees and more) in most in some cases, the lands belonging to small households are cultivated along the slopes, thus spreading the layer of fertile soil. At the same time, lands located on slopes with an inclination greater than 10 degrees are not excluded from the circuit of intensive processing. There is a danger that for 5-6 years a considerable part of the lands will be completely degraded and removed from the agricultural circuit. In the total area of agricultural crops, plowing prevails - 46.7%, including 10.8% grass crops and 42.5% fallow. Of the perennial plantations, orchards and vineyards predominate. Some households have 30-40 ha of arable land and 10-20 ha of perennial plantations on degraded soils. Threshold systems are not created in the complex with the planting of forest protection sheets. The gullies are not leveled, the surfaces subject to landslides are aired and the ravines are not covered with vegetation. Scientifically based crop rotations are not respected, basically they have switched to simple crop rotation. On the privatized lands, corn is mainly grown in monoculture. The massive cultivation of cereals and technical plants is determined by market demand, and is quite difficult to influence.

The management of land resources includes a wide spectrum of public relations, social, economic, ecological and other types of

management. For this reason, the management of land resources represents a systematic, conscious and strictly directed action of the state and society regarding land relations. Land resources represent not only the territorial, spatial and natural basis for the ethnic location of a society, but also an object of ecological and economic management.
The main conclusions from Figure 3 regarding the land structure by microzones are the following: - over 50% of the district's land fund is concentrated in the Nisporeni and NordEst microzones, which also own over 46% of agricultural land and approx. 60% of the forestry fund; - in the structure of agricultural holdings, the lowest share of multi-year plantations (vineyards and orchards) is in the Frontier and in the North-West; - in the northern part of the district, most of the reserve lands are located; - the water basins are distributed almost evenly in three microzones – Nisporeni, Frontiera and North-West, in the other two microzones the share of water basins is lower; - the smallest forested area is at the Border and along the district segment of the Chisinau-Leuşeni highway.

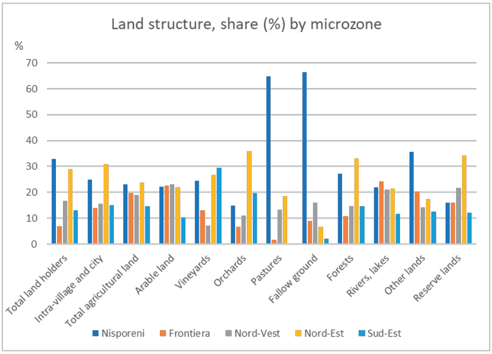

Figure 3. Representation of the land structure, share (%) by microzone of the Nisporeni district

The problems and discrepancies of the microzones represented in Figure 3: - the Nisporeni microzone (3 localities, including one city) occupies an extensive area, including the built-up area, it concentrates approx. 1/4 of the vineyards and orchards of the district, as well as over 27% of the forests, however it has a large number of fallow land and relatively small land reserves; - the Frontiera microzone (6 communes, 6 localities) is small, with a low degree of afforestation and a small share of perennial plantations; - the North-West microzone (4 communes, 11 villages) is small, has the highest share of arable land, but with the lowest degree of perennial plantations (vineyards, orchards) and forest areas; - the North-East microzone (6 municipalities, 10 villages) is the largest and occupies approx. 1/3 of the territory of the district, including the built-up area, has the most vineyards and orchards, the highest degree of forestation, but also the smallest area of water basins; - the South-East microzone (5 municipalities, 9 villages) is the smallest in the district, including built-up area, with few water basins and reserve lands, instead with the highest gad coverage with perennial plantations and the lowest share of fallow land.
Economic agents in agriculture (Figure 4) are largely peasant households, relatively homogeneously distributed on the territory of the district, but with a greater share in the North-East and the Frontier (approx. 50% of peasant households). Most of the agricultural cooperatives and associations of peasant households are in the Nisporeni microzone. This fact denotes the need to strengthen the capacities of small farmers in larger households towards the outskirts of the district

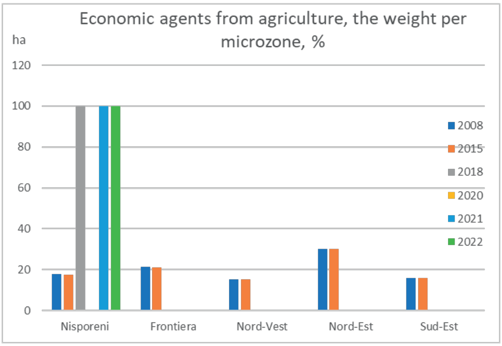

Figure 4. The division of economic agents from agriculture, the weight per microzone, % per year in the territory

Agriculture has an important role in the economy of the district. Agricultural lands occupy 48.0% of the total area of the district. The private sector owns most of the agricultural land and also provides most of the agricultural production. According to the statistical data for the district, in 2012 the agricultural production (in current prices) amounted to 571.7 thousand lei, but this amount has increased drastically nowadays. Agricultural production per capita is 4.8 thousand lei. The average soil quality is 52 degrees. The anti-erosion protection system consists of 435 ha of protective forest strips.

The total area treated with chemical preparations is 11342 ha (approx. 4.1% of the total). In 2012, 210 tons of mineral fertilizers were introduced into the soil on 1620 ha (7.8% of the total), or 129.6 kg/ha.

The structure of agricultural land is relatively stable. The largest share is held by arable land - 71.8% of the total area of land intended for agriculture. Arable land makes up 19,578.2 ha (according to agricultural statistics), or 68.2% of the total area of land intended for agriculture.

Grapevines are grown on 4.6 thousand ha, orchards on 2.5 thousand ha. The level of planting vines is much higher than the level of planting orchards. The dynamics of agricultural holdings registers a slight increase due to the reserve fund of the municipalities.

The total area of eroded and damaged agricultural land is 22,934 ha, including by category - slightly eroded agricultural land - 10597 ha, medium eroded - 5312 ha, heavily eroded - 4562 ha, another category of land is landslides that occur from due to deforestation, earthquakes and heavy rains in the district are present on an area of 2025.27 ha, the formation of ravines constitutes an area of 437.39 ha.

Agricultural production is carried out at local, district markets and in Chisinau. A small volume of products is exported fresh abroad. Most of the peasant households satisfy only the family's own needs. There are no large enterprises for the collection and processing of agricultural production in the district, with the exception of wine-growing and plum drying enterprises. We need cooperatives, commercial companies, which would collect, process and deliver the agricultural production harvested by the land owners in the localities of the district.

The share of people employed in the industry is approx. 13.8%, but with some fluctuations depending on the situation on the regional markets. There are 64 enterprises processing local products in the district. At the same time, the Nisporeni district is characterized by a low level of development of the industrial sector. The district's industrial sector is represented by the manufacturing industry (91.8%, with sales revenues of 100,151.6 thousand lei) and light industry (8.2%, sales of 3.4 million lei) specialized in the manufacture of clothing. The share of people employed in the industrial sector in the district is 11.8%. The average number of employees in industrial enterprises is over 700 people, with an average monthly salary of 3183.3 thousand lei. The private sector in industry a
ensured 90.5% of the total production volume, manufactured in the district; the mixed one – 9.4%.

The main achievements in the field of soil protection were the following project carried out with the help of IFAD, namely the project called „Works to create the protective forest plantation within the Seliște town hall, Nisporeni district", which was also carried out with the help of IFAD, works of planting were carried out in an area of degraded land, according to the contract the planting works were carried out by the Forestry Enterprise „Nisporeni - Silva" with an area of 10 ha.

The main capital investment achievements provided for the protection of soils and the implementation of projects in this field, including the funded ones, are the massive planting within the „A tree for our survival" campaign during the given period, a number of 29315 shrubs were planted on an area of approximately 36, 5 ha with the aim of expanding forestry crops and reducing eroded land surfaces and with an increased degree of landslides. The given actions contribute to the regeneration of forests and the expansion of areas with forest vegetation as well as to the improvement of environmental conditions.

During 2022, in the Nisporeni district, the following violations were detected that caused damage to soil resources, namely the case of stubble burning in the village of Grozești, which through his actions the fined villager caused damage to land resources following the burning of vegetable waste in the open field of 2500 lei and paid in full.

The problem of soil erosion and degradation are increasing, while efforts aimed at restoring the fertility and productive functions of the soil are not producing the expected effects, which causes an increasing impact on other sectors of the environment, as well as on human health and animals. Ecological and economic advantages regarding the implementation of environmentally friendly agricultural practices.

The creation and establishment of grassy canals along the natural path of the drains has the following advantages: - they can be created with the farm's own equipment and forces; - protects the drainage path against erosion and the formation of ravines; - acts as a filter, absorbing part of the chemicals and nutrients from the draining water; - provides shelter for birds and small animals; - allows to obtain green and fibrous fodder for feeding animals.

The priority would be the rotation of agricultural crops (rotation). This agricultural practice has a favorable influence on the environment, namely: - it reduces consumption for the procurement and application of pesticides by naturally interrupting the growth cycles of weeds, insects and the development of diseases; - the pasture/hay crops (perennial grasses and legumes) and thatched cereals, which accumulate sufficient plant residues in rotation, considerably reduce soil erosion; - crop rotations that include grasses (crested pyrites, barberry, golomat, orchard grass) and perennial legumes (alfalfa, clover) protect water quality by preventing the infiltration of nutrients and chemicals into water sources.

Irrigation of agricultural crops has become a necessity, given the conditions in which drought and lack of precipitation are increasingly common phenomena. The success of farmers in the Nisporeni district depends a lot on the availability, condition and functionality, as well as the development prospects of the irrigation infrastructure. The irrigation system in the village of Grozești, which covers an area of about 1000 hectares for agricultural producers in the villages of Grozești, Bărboieni and Soltăneşti in Nisporeni district, was renovated within the Compact Program of the US Government. For the reconstruction of this system, the Compact Program invested more than 8 million USD. It was put into operation in 2015, and in 2016 the activities started.

The high instability of agricultural production in the district is a consequence of poorly developed instruments for mitigating risks related to climate conditions, including insufficient access to irrigation, the low level of application of modern agricultural technologies (drought-resistant varieties, hail protection tools) and the lack of innovative insurance schemes in agriculture, such as the climate index insurance programme. Another reason for the slowdown in agricultural production is the economic crises, which lead to an increase in the prices of inputs (for example, fertilizers, fuel, cars), causing difficulties for agricultural producers.

Soil degradation is another factor that determines the low profitability of the agricultural sector, as well as the dominant position of low-value crops in agricultural production at the expense of high-value crops (fruit growing, vegetables, viticulture). A significant part of the agricultural production is represented by the 4 main products: cereals, grapes, legumes and fruits.

The structure of the sown lands for the year 2022, ha: cereal crops - 1061 ha; corn - 8560 ha; sunflower - 2590 ha; grapes - 4689 ha; fruits - 2690 ha; vegetables - 175 ha.

It is obvious that cereals (including wheat, barley, corn and sunflower) are the first on the respective list, due to the large share of arable land - about 40% of the total sown area. Reasons why producers focus on grain include large-scale mechanization, relatively low capital requirements, low labor intensity, reliable markets and profit opportunities, and the limited need for irrigation—all of which show that large agricultural enterprises are the dominant form of organization.

The emphasis on the cultivation of agricultural products with high added value (grapes, vegetables, fruits) is imperative for the Nisporeni district. recently walnuts are cultivated in the district. This product has access to EU markets as well as to other markets. The attractiveness of this sector is largely explained by the mild climate, resistance to pests, diseases and droughts, as well as limited resources for maintenance.

<u>Priority measures and actions for the Nisporeni district</u>: - optimizing the exploitation of land resources by improving the cadastral system, consolidating land, repairing access roads to land located at great distances from the locality,

modern land processing technologies; - attracting priority investments in the planting of vines and intensive orchards (at the Border and in the North-West) and for the highly technological processing of agricultural products in the economic growth centers of each micro-region; - the revitalization in the border area of vegetable growing, viticulture, fruit growing, the animal husbandry sector, where traditionally it was developed, and currently these branches are practically destroyed. In order to attract investments in agriculture in the Prut area, where the soils have high fertility, it is necessary to build the road Bărboeni-Bălăureşti with a length of 12.7 km and Bălăureşti-Nisporeni - 12.1 km; - combating soil erosion on disadvantaged lands (approx. 70% on slopes) with modern agro-pedological technologies (Nisporeni, North-West).

CONCLUSIONS

The main branches of the economy of the Nisporeni district are: agriculture and the processing industry of agricultural production, services and trade. The degree of diversification of the economy is extremely low, especially through small trade and service enterprises.

The Nisporeni district is predominantly agricultural based on small individual agricultural owners and a limited number of enterprises that process local production up to the finished product.

The basic activity in the economy of the district is agriculture, but in most cases the entrepreneurs and peasant households practicing agriculture do not respect the ecological and agrotechnical rules, most of the agricultural households are located on steep slopes (up to 25 - 30 degrees and more) in most in some cases, the lands belonging to small households are cultivated along the slopes, thus spreading the layer of fertile soil.

Of the perennial plantations, orchards and vineyards predominate. Some households have 30-40 ha of arable land and 10-20 ha of perennial plantations on degraded soils. The structure of agricultural land is relatively stable.

The total area of eroded and damaged agricultural land is 22,934 ha, including by category - slightly eroded agricultural land - 10597 ha, medium eroded - 5312 ha, heavily eroded - 4562 ha, another category of land is landslides that occur from due to deforestation, earthquakes and heavy rains in the district are present on an area of 2025.27 ha, the formation of ravines constitutes an area of 437.39 ha.

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INFLUENCE OF DIFFERENT AGRICULTURAL MANAGEMENT PRACTICES ON SOIL MICROBIOME

Andrei-Mihai GAFENCU, Andreea-Mihaela FLOREA, Florin-Daniel LIPȘA, Eugen ULEA

"Ion Ionescu de la Brad" Iasi University of Life Sciences, 3 Mihail Sadoveanu Alley, 700490, Iasi, Romania

Corresponding author email: agafencu@uaiasi.ro

Abstract

Microbiomes are the collection of all microbial inhabitants of a given system. At the level of the soil, the microbiome includes four major groups of microorganisms: bacteria, fungi, archaea, and protozoa, as they are the main organisms for essential soil processes such as nutrient cycling, decomposition of organic matter, and plant growth promotion. A healthy soil microbiome is essential for sustainable agriculture and the overall health of terrestrial ecosystems. Some agricultural management practices, i.e. irrigation, can have a significant impact on the soil microbiome. The quality and quantity of irrigation water can affect the abundance and diversity of microorganisms in the soil. For example, excess irrigation can lead to waterlogging, which can create anaerobic conditions that favour the growth of certain types of bacteria. On the other hand, irrigation water in the right amounts can have a positive impact on the soil microbiome and promote plant growth. To test this hypothesis in the agricultural year 2022, a research was carried out on maize and soybean grown under irrigated conditions, to analyse the variations of microbial density during the vegetation period.

Key words*: soil microbiome, soil bacteria communities, soil microbiota.*

INTRODUCTION

Soil microorganisms are essential to the environment, playing an important role in keeping the environment healthy and productive (Chaparro et al., 2012). One of the main activities carried out by microorganisms in the soil is represented by the decomposition of organic matter (Xu et al., 2015). By carrying out this process in the soil, nutrients are released which are then available to be used by plants, but also by other microorganisms (Osorio Vega, 2007; Jacoby et al., 2017). Some soil microorganisms are involved in nitrogen fixation, a process by which atmospheric nitrogen is converted into a form that plants can use. This is important because plants need nitrogen to grow and develop (Mus et al., 2016; Soumare et al., 2020). In addition to the role they play in fixing nitrogen and bringing it into forms easily accessible to plants, microorganisms also play an important role in the solubilisation of phosphorus (Alori et al., 2017; Ingle et al., 2017; Rawat et al., 2021) and potassium (Sharma et al., 2016; Bahadur et al., 2016; Ahmad et al., 2016). Also, soil microorganisms are essential to the environment due to their role in promoting plant growth and various biophysical processes (Prasad et al., 2015; Basu et al., 2021). Soil microbiome exhibit natural seasonal dynamics and their biodiversity, abundance, and functions are influenced by various factors including crops, climatic conditions, and agricultural management practices, i.e. tillage systems, fertilizers, pesticides and irrigation regime (Entry et al., 2008; Geisseler et al., 2014; Gafencu et al., 2021).

The objective of this paper is to test the hypothesis that agricultural management practices (i.e. use of irrigation water) lead to changes in the composition and abundance of the soil microbial community.

MATERIALS AND METHODS

The field experiment was conducted at Agralmixt S.A. farm, in North-Eastern Romania, Iasi county, Andrieseni village ($47^0$34'9" N, $27^0$20'38" E, 60 m above sea level). Prior to the experiment establishment, the tillage was carried out by using a scarificator at ~30 cm depth, then was followed by the preparation of the germinal bed by passes with the seed bed combination system. Soybean (*Glycine max* L.) and corn (*Zea mays* L.) were the crops taken for investigation. Cultivation technology was represented by conventional technology, chemical fertilizers were used and, applied before soil tillage. During the vegetation period, plant protection products were also applied.

During corn and soybean vegetation period a quantity of 200 l/m^2 water was applied to cover the needs of the crops.

The regional climate is a typical temperate continental climate. The average annual temperature is ~9.5^{0}C, with extremes ranging from 40.0^{0}C to -35.0^{0}C (Gafencu, 2019). The annual precipitation is ~520 mm. Significant deviations from the long-term average precipitation and temperature have been observed in the last 10 years. The soil texture is primary clay-loam and the natural vegetation of the site is silvo-steppe.

The soil samples were taken from two agricultural plots cultivated with maize (*Zea mays* L.) and soybean (*Glycine max* L.).

From each plot, 10 points were randomly selected from where soil samples were taken, the entire area of the plots being covered.

Soil samples were taken from three different depths: 7-10 cm, 10-20 cm, and 20-30 cm, using sterile sampling tools. The soil samples were taken six times during the vegetation period of the maize crop, between May and October 2022. Soil samples were transported to the microbiology laboratory, stored overnight at 4^{0}C, dried at room temperature and sieved (2-mm mesh) prior to further use in the experiment.

The total number of bacterial colony forming units (CFUs) was determined by serial dilution method and plating into nutritive media on PDA (Potato Dextrose Agar medium) in different compositions: classic and with streptomycin. Streptomycin (35 mg·L^{-1}) is an antibiotic that has been used to inhibit the growth of Gram negative bacteria.

From the collected samples, successive dilutions were made in sterile water, using a dilution coefficient in the rate of 10 (dilutions 10^{-1}, 10^{-2}, … , 10^{-6}). By this technique a series of dilutions are obtained in which the number of germs decreases in arithmetic regression. To prepare these dilutions, 9 ml of double-distilled water sterilized at 120°C for 30 minutes was distributed in sterile tubes of 15 ml capacity. One g of soil was weighed onto a sterile watch glass and placed in the first dilution tube. After vigorous stirring for five minutes a 10^{-1} (1/10) dilution was obtained. From this dilution, 1 ml of suspension was transferred to another test tube with 9 ml of sterile water, obtaining the dilution 1/100 (10^{-2}). The same way the other dilutions were obtained. 1 ml of suspension from each dilution was introduced into a Petri dish (Gafencu et al., 2022). After 24 h at 28^{0}C, the colonies were counted. Using Scan 1200 colony counter, the bacterial colonies that formed on the medium were counted. To determine the number of bacteria in one gram of soil, the number of colonies that developed in Petri dish was multiplied by the inverse value of the dilution. The count result was related to the dilution used and the final result was expressed in colony forming units (CFUs) per 1 g of soil.

RESULTS AND DISCUSSIONS

Some recent studies on the impact of irrigation water on soil microbiota have shown that water has a positive effect on the structure, abundance and activities of soil microbial communities (Entry et al., 2008).

Following the changes in the abundance and structure of soil microbial communities during the growing season of maize (Figure 1) and soybean (Figure 2) it is observed that the dynamics of bacterial communities follows an increasing trend from May to October. Analysing the results, it can be seen that from the time of sowing until May, the numerical density of bacteria decreased in the case of both crops. This was due to the fact that in the first part of the vegetation period the microbial community was influenced by the phytosanitary treatments applied to the crops.

After the sampling carried out in May, the crops were irrigated. Starting from this moment, in the case of maize crop, the bacterial abundance increased until September, the month in which the last irrigation was applied. In October, there was a slight reduction in the number of bacteria in the soil. In addition to the evolution of the abundance of bacteria in the soil, the changes that take place at different depths were also analysed.

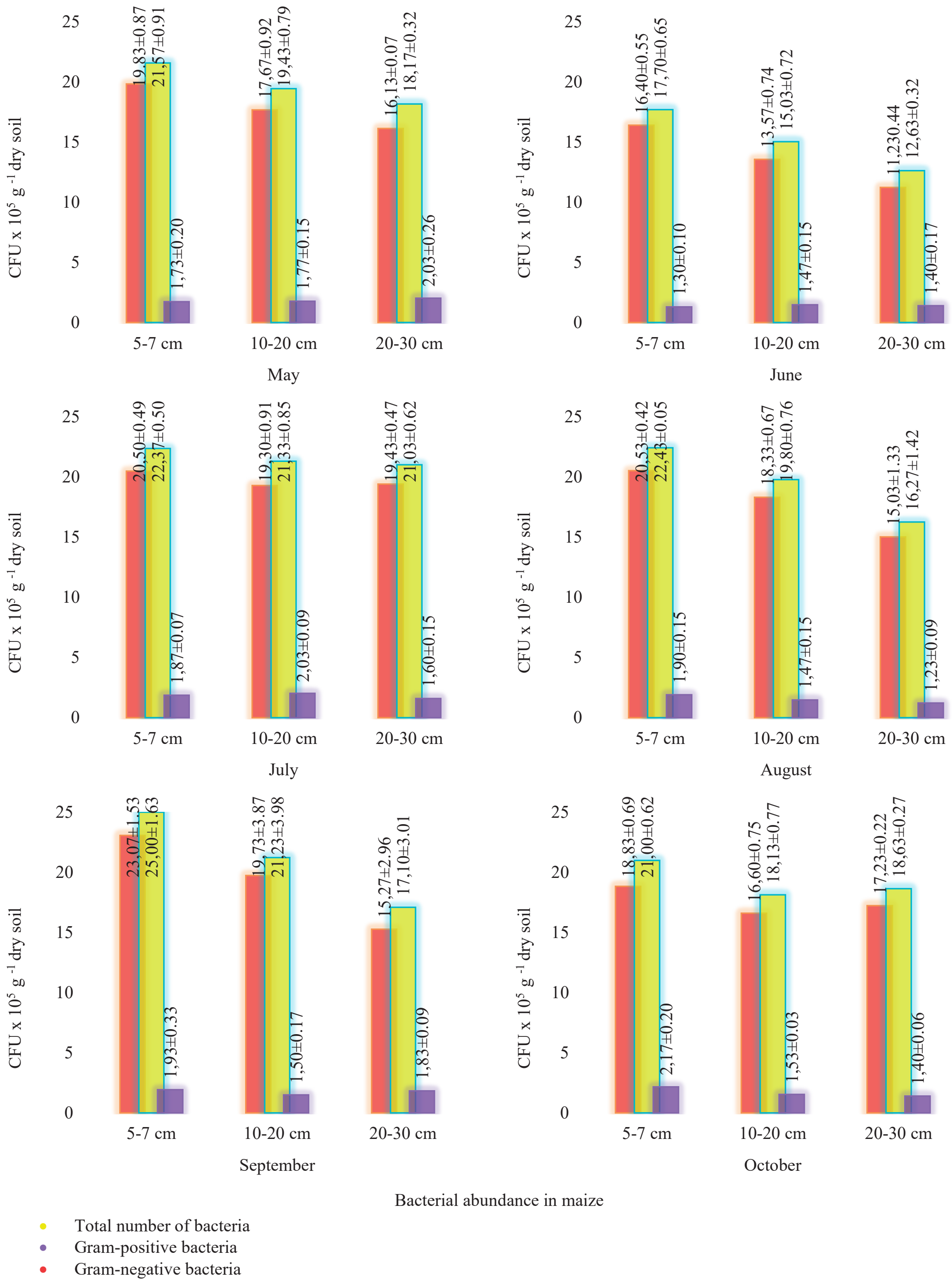


Figure 1. Changes of soil bacterial counts and incidence during the maize growing season

Under this aspect, it can be observed that at 20-30 cm depth, in May there were 18.17 ± 0.32 CFU x 10^5 g^{-1} dry soil. In June, the number of bacteria decreased, and starting from July, when the peak of the increase in the abundance of total number of bacteria was observed, and until

October, the number of bacteria followed an increasing trend. In the case of the soybean crop, the total number of soil bacteria fluctuated during the growing season.

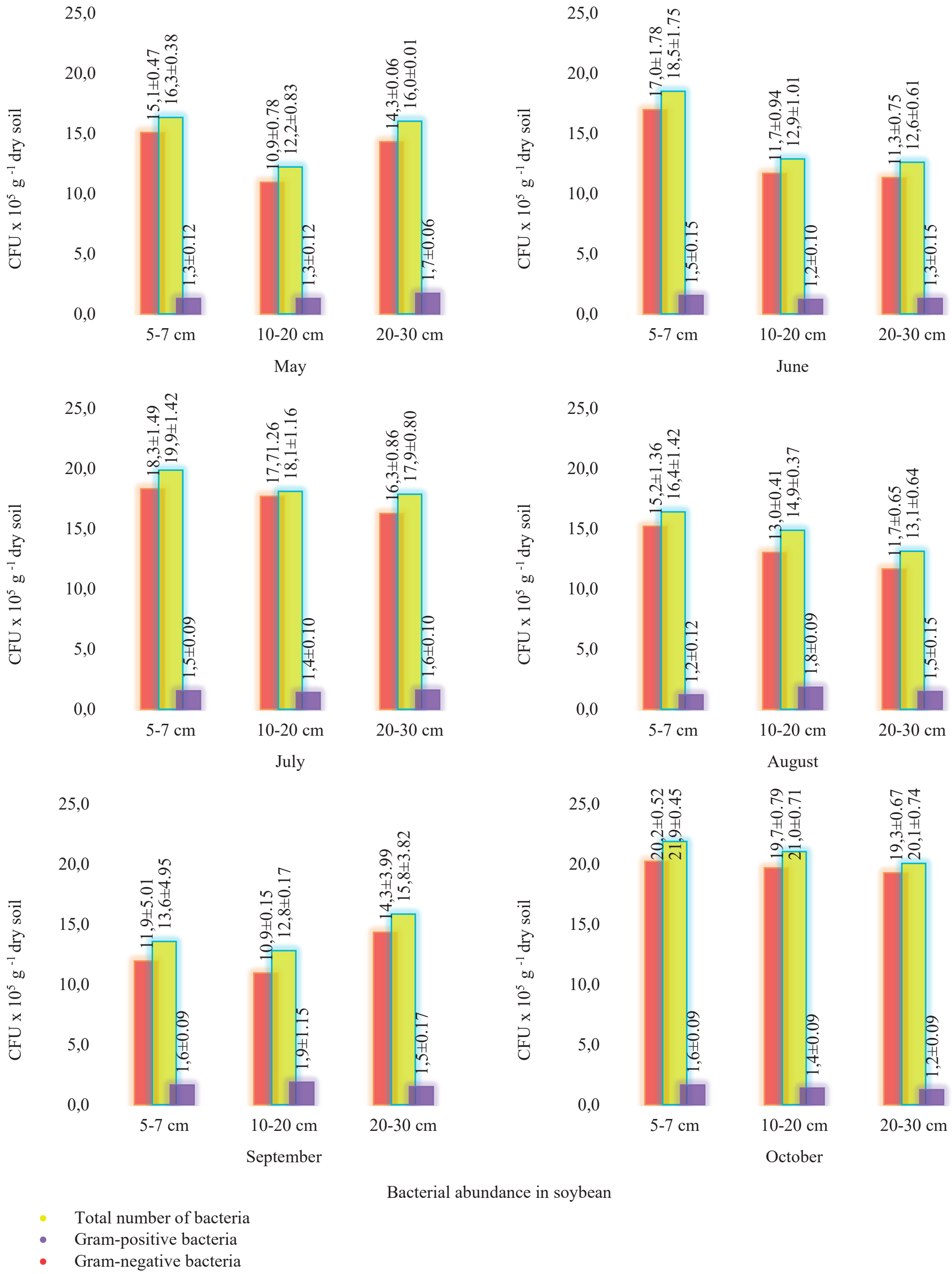
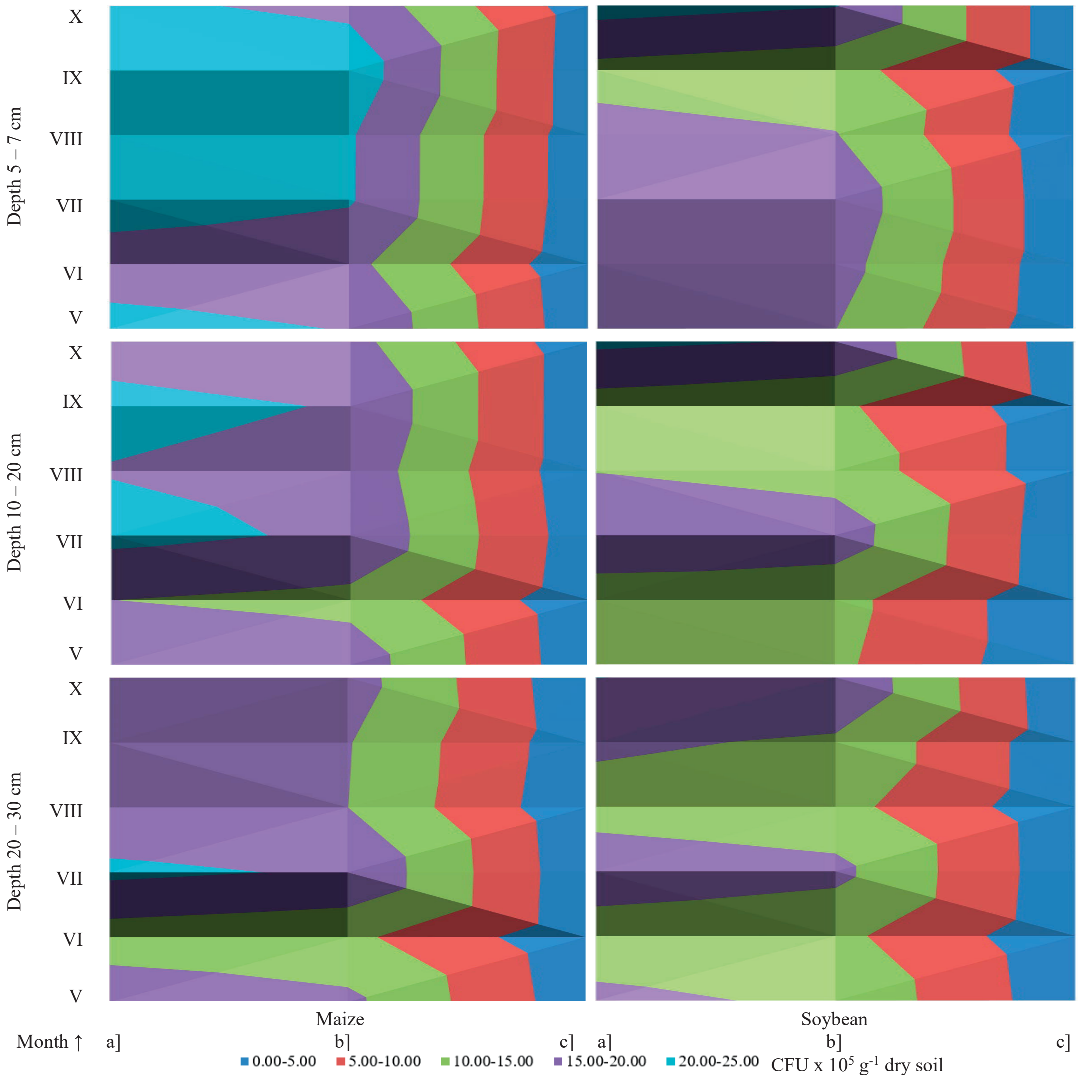


Figure 2. Changes of soil bacterial counts and incidence during the soybean growing season

After the sampling carried out in May, the soybean crop was irrigated and the numerical density of the bacteria increased until July. In August, the total number of bacteria decreased, then increased in September and October, when the highest value was recorded, i.e. 21.90±0.45 CFU x 10^5 g^{-1} dry soil. Both in maize and soybean crops the total number of bacteria increased till the end of the vegetation period at 10-20 cm depth, respectively 20-30 cm. Analysing the effect of seasonal variation on soil bacterial communities, it was observed that Gram-negative bacteria dominated soil bacterial communities (Figure 3).



a] Total number of bacteria (CFU x 10^5 g^{-1} dry soil); b] Gram-negative bacteria (CFU x 10^5 g^{-1} dry soil); c] Gram-positive bacteria (CFU x 10^5 g^{-1} dry soil)

Figure 3. Differences between the abundance of soil bacterial communities during the vegetation period in maize and soybean crops

Analysing the evolution of the numerical density of bacteria, it was observed that in the case of maize crop, the abundance recorded higher values compared to the soybean crop. Regarding the ratio between Gram-negative bacteria and Gram-positive bacteria, it was observed that this ratio remained unchanged throughout the vegetation period, a situation encountered in the case of both crops.

CONCLUSIONS

Following the study, changes were observed in the abundance and structure of soil microbial communities. In the 5-7 cm soil layer, during the entire vegetation period, the highest values of the numerical density of the bacterial communities were recorded. This trend is typical, representing the normal evolution of bacterial communities in the soil, from the time of sowing to the time of crop flowering, the growth being relatively constant. An important aspect is represented by the fact that the microbial communities underwent changes in the 10-20 cm and 20-30 cm depth ranges. This is important because increasing the density of microbial communities at greater depths can have a positive effect on soil and soil processes, as well as plant health and development.

These results represent an important starting point for future research on the influence of irrigation water on soil microbiota.

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COMPACTION OF ARABLE CHERNOZEMS: PEDOFUNCTIONAL AND AGROTECHNOLOGICAL CONSIDERATIONS

Gheorghe JIGAU[1], Tatiana CIOLACU[2], Boris TURCHIN[1], Sergiu DOBROJAN[1], Galina DOBROJAN[1]

[1]State University of Moldova, 65 Cogalniceanu M. Street, Chisinau, Republic of Moldova
[2]Institute of Ecology and Geography, 1 Academiei Street, Chisinau, Republic of Moldova

Corresponding author email: gheorghe.jigau@gmail.com

Abstract

The contemporary evolution processes of arable chernozems lead to the modification of the indices of the agrogen layer settlement state materialized in the change of the ratio between the mass and the volume of the soil phases in the evolutionary-genetic sequence: compaction → overcompaction → settling → vertisolation. It is favored by the high proportion of interaggregate pores, humus loss, disaggregation and destructuring of soil, the increased content of fine clay (< 0.001 mm) in the physical clay (< 0.01 mm), the low degree of saturation of physical clay with humus, the high proportion of smectite-montmorillonites in the composition of clay minerals of the fine clay fraction. The modification of the settlement indices conducts to the establishment of some regimes and processes in soils with an impact on the direction and intensity of the evolution processes of chernozems in arable regime, with the involvement of new mechanisms and processes uncharacteristic for native chernozems: compaction and overcompaction of structural aggregates, eluviation of finely dispersed clay from the arable layer to the sub-arable layer, reduction of the intensity of the chernozemous process. These lead to the detachment of the arable chernozems from the native ones and implies the need to establish a new management paradigm of arable chernozems in the framework of bioremedial adaptive-landscape technologies.

Key words: *compaction, differential porosity, humus loss, vertisolation, settling.*

INTRODUCTION

The multiple pedological investigations carried out in the Pre-Danubian area since the nineties of the 19th century have shown, unanimously, that the structure of its soil cover is dominated by leached and typical chernozems with moderate and low humus content, a medium-fine granulometric composition (clayey loamy and loamy clayey) with an increased degree of potential fertility. The vertiginous development of intensive agriculture in the post-war period attracted their inclusion in the agricultural circuit, the degree of their utilization in agriculture reaching more than 86% at the end of the nineties of the last century. The substitution of natural biocenoses with agrophytocenoses led to the significant modification of all regimes (hydric, thermal, aeration, aerohydric, hydrothermal) and processes (formation and accumulation of humus; aggregation-structuring; biogenic accumulation) responsible for chernozemic pedogenesis, including the entire complex of pedo-biological processes, which determine the peculiarities of the biological circuit of substances and the functioning of the soil ecosystem (Jigau et al., 2021). In addition, a series of modification-transformation processes of all components and phases of chernozems were established in the soils.

According to the calculations, even in the first 5-10 years of the restructuring of the soil ecosystem included in the agricultural circuit, an unidirectional trend of mineralization of organic substances, including humus, was established in them. The losses of humus from these during the last 100 years made up more than 25% of the initial reserves (Ursu, 2011). The composition of the system of humic substances also suffered significant changes, manifested in its fulvatization.

Under the conditions of significant changes in the pedofunctional regimes (aerohydric, hydrothermal, redox) within the humification process, complex humic substances characteristic of the chernozemic process are no longer produced. As a result of the intensification of biogenic alteration processes in the arable layer of clay minerals from the group of hydromics, perceptible mineral changes are attested in it (Tshovrebov et al., 2017).

The specified changes attracted, by themselves, the establishment in the arable chernozems of a new mechanism of spatial organization of their organo-mineral component, which involves the disintegration-metaaggregation of the chernozemic structure with the development of agrogenic aggregate formations (Jigau et al., 2021).

The inter-determined and interdependent modification of the humification and aggregation-structuring processes led to the disruption of the functionality of the pedofunctional system [humic system ↔ aggregate system] manifested in the change in the state of soil settlement, the integrative indices of which are the bulk density (compaction) and the pore space of soils.

Despite the multiple researches dedicated to soil compaction in Soil Physics, until now no comprehensive concept has been formulated regarding the factors and processes that determine it. It is certain that soil compaction is a phenomenon inherent to the inclusion of soils in the arable circuit (Jigau and Nagacevschi, 2006).

In this sense, in the scientific literature soil compaction is examined through the lens of its perception as a result of the change in the way of organizing their solid component under the action of intensive works and the exaggerated traffic of machines and agricultural aggregates on the land surface.

In this paper, soil compaction is examined through the lens of disrupting the functionality of the pedofunctional system [bioenergetics system ↔ aggregate system].

MATERIALS AND METHODS

In this paper, soil compaction is examined as a phenomenon of change in the ratio between mass and volume of soil phases through the prism of the energetic concept of physical state (Voronin, 1990) and the thermodynamic concept of soil quality (Смачин, 2021). In accordance with these and through the prism of the concept of hierarchy, interdependence and interaction of soil properties, regimes and pedogenetic processes (properties ↔ regimes ↔ pedogenetic processes) within anthropo-natural pedogenesis, compaction is a complex polygenetic process inherent in its evolution and represents an early phase within of the evolutionary-genetic chain “compaction → overcompaction → settling → vertisolation” of changing the ratio between the mass and the volume of the soil phases manifested in various ways of structural-functional organization of the soil mass.

The realization of the mentioned evolutionary-genetic chain is favored by the increased compaction-slitic potential determined by the medium-fine granulometric composition (clayey loamy and loamy clayey), the increased content of fine clay (< 0.001 mm), the increased degree of unsaturation of the physical clay (< 0.01 mm) with humus, illite-montmorillonite composition of fine clay.

The driving force of compaction and its evolution is a series of agrogenic processes (humus loss, hydrophilization of the organo-mineral matrix, disaggregation-destructuring of the soil mass, mineral transformations in the mineralogical composition of the fine clay fraction as a result of the intensification of biological alteration processes in the arable layer), the changes in pedogenetic and pedofunctional regimes induced by climatic instability, the mechanical pressures exerted on the soil by machines and agricultural aggregates.

In accordance with the work concept presented above, the activities in the laboratory included:

1. Determination of the dynamics of the bulk density in a multi-year regime - the N. Kacinski compactimeter method (Jigau, 2006).
2. Determination of the density and porosity of aggregates 5-1 mm - the Lâtaev method (Jigau and Nagacevschi, 2006).
3. Determination of the dynamics of the structural-aggregate composition - the Savvinov method (Jigau and Nagacevschi, 2006).
4. Determination of the mineralogical composition of the fine clay fraction (< 0.001 mm) - diffractometric method.
5. Calculation of differential porosity - suction curve method.

RESULTS AND DISCUSSIONS

With reference to agricultural soils, two categories of compaction are more frequently examined in the scientific literature: a) primary, b) secondary (agrogenic). Primary compaction is examined as a trait inherited from the parent rock with some modifications induced by the pedogenesis process. Agrogenic compaction, more often, is examined simplistically through the lens of the utilitarian approach in relations with agricultural aggregates and cultivated crops. In this sense, soil compaction is defined as a process caused by excessive and irrational traffic on the land for agricultural works, especially in inadequate moisture conditions, as a result of which the apparent density values increase above the optimal values for agricultural crops, but remain in the range of values within which decompaction is ensured by self-loosening. Unlike the primary compaction in the soil profile, this is limited only to the agrogenic layer within which two layers with different compaction mechanisms are clearly outlined: a) arable and b) sub-arable.

The compaction of the arable layer is a very common phenomenon, which occurs during intensive tillage of the soil and is determined by its degradation-metastructuring: tillage → substitution of biocenoses with agrophytocenoses → destruction of the soil layer → degradation of humic detritus → degradation of the humic system → destructuring-metaaggregation-compaction.

The realization of this mechanism does not involve the formation of new swelling or agglutinating mineral formations and is determined by the modification of the way the solid constituents are placed. The effects of compaction are intensified by the pressure exerted by the mass of agricultural machines.

The phenomenon of compaction of the sub-arable layer is usually known as "plow sole", although its origin is not only caused by plowing works. The sub-arable layer, unlike the arable layer, is more easily compacted because it is wetter and is characterized by a more rigid settlement under the action of the pressure exerted by the mass of the overlying layer, the lower content of organic matter, less structured and the structure is less stable. In it, the compaction is intensified by the metaaggregation processes under the influence of the mechanical forces exerted by the agricultural aggregates. As a result, the total volume of the porous space is significantly reduced in the sub-arable layer due to the interaggregate pores with the formation of prismoid-nut-shaped aggregate formations.

The clearly described mechanisms are outlined in the agrophysical profile of arable chernozems (Table 1).

Table 1. Dynamics over time and on the profile of the bulk density of the typical loamy chernozem with low humus content on clay loam in the period 2010-2019 (LLC VINDEX-AGRO, Orhei district)

Horizon	Depth, cm	2010		2013		2016		2019	
		1*	2**	1*	2**	1*	2**	1*	2**
Am/Aar	0-10	1.02	1.26	1.00	1.23	0.98	1.21	1.01	1.25
	10-20	1.05	1.24	1.03	1.25	1.02	1.26	1.03	1.20
	20-30	1.14	1.24	1.14	1.28	1.13	1.24	1.11	1.24
Am Bm	30-40	1.18	1.33	1.20	1.35	1.12	1.34	1.17	1.33
	40-50	1.22	1.36	1.25	1.35	1.22	1.37	1.22	1.35
Bma	50-60	1.28	1.34	1.27	1.35	1.27	1.35	1.25	1.34
	60-70	1.30	1.35	1.32	1.34	1.30	1.34	1.33	1.34
	70-80	1.29	1.33	1.33	1.33	1.32	1.35	1.31	1.36
B2ca	80-90	1.32	1.33	1.33	1.35	1.31	1.34	1.30	1.34
BCca	90-100	1.33	1.32	1.32	1.34	1.30	1.32	1.31	1.32
Cca	110-120	1.30	1.30	1.31	1.33	1.31	1.33	1.32	1.33

1* - nativ
2** - arable

From Table 1 we can see that the chernozems systematically subjected to the works are detached from the unworked chernozioms by higher values of the bulk density in the arable layer (0-30 cm). Compared to the native chernozems in the arable layer of the arable chernozems, the bulk density values are by 0.1-0.2 g/cm^3 higher and indicate the establishment of the compaction process in them. At the same time, during the observation period, the bulk density values in it remain in the range of optimal values within which the return to the balanced values is ensured through self-loosening and self-decompaction.

In the native chernozems, the bulk density values in the 30-60 cm layer increase slightly but remain in the range of optimal values (< 1.30 g/cm). The slight but noticeable increase in the values of the bulk density in this layer of the native chernozems is caused by the reduction of the role of the root mechanism in the self-loosening of the soil. In the same layer of arable chernozems, during the entire period of observations, the bulk density shows values characteristic of the range of critical values for chernozems (1.3-1.4 g/cm^3), which indicate the overcompaction process. In it, the self-decompaction processes ensure only the partial decompaction of the soil mass, in connection with which the bulk density values are established at a higher, but relatively stable, level. This implies the conclusion that the overcompaction of the 30-60 cm layer is only partially caused by the mechanical forces originating from the traffic on the soil surface and its work and is achieved due to the reduction of the interaggregate pore volume. At the same time, in the sub-arable layer, a reduction in the volume of the aggregate pores can be observed as a result of their clogging with finely dispersed particles leached from the arable layer (Tables 2, 3).

Table 2. The density and porosity of the 5-1 mm aggregates of typical weakly and moderately humic arable chernozems under conditions of various tillage systems (agrogenic layer 0-50 cm)

Soil, Crop	Aggre-gate diameter, mm	Depth, cm, density (g/cm3) and porosity (%) of aggregates															
		Conventional technology								Adaptive-landscape technology							
		0-15		15-20		25-30		45-30		0-15		15-20		25-30		45-30	
		1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Typical chernozem with low humus content, winter wheat	5-3	1.59	38	1.63	37	1.69	35	1.64	36	1.54	38	1.57	39	1.63	37	1.60	39
	3-2	1.61	37	1.68	35	1.75	32	1.72	34	1.57	38	1.63	37	1.70	34	1.66	36
	2-1	1.64	36	1.73	33	1.86	28	1.80	31	1.60	37	1.69	34	1.79	31	1.74	33
Typical chernozem with low humus content, winter barley	5-3	1.57	38	1.60	38	1.70	34	1.67	36	1.53	40	1.57	39	1.61	38	1.57	40
	3-2	1.62	36	1.70	34	1.88	27	1.79	31	1.58	38	1.62	37	1.73	33	1.69	35
	2-1	1.66	35	1.83	29	1.94	25	1.87	28	1.65	35	1.70	34	1.80	31	1.73	34
Typical chernozem with medium humus content, rape	5-3	1.58	38	1.63	37	1.71	34	1.68	36	1.53	40	1.60	38	1.68	38	1.60	39
	3-2	1.65	35	1.76	32	1.85	29	1.81	31	1.57	38	1.63	37	1.71	34	1.58	39
	2-1	1.72	33	1.88	27	1.97	24	1.92	26	1.67	36	1.74	34	1.77	32	1.71	34

Table 3. Differential porosity of typical chernozems with moderate humus content and different degrees of agrogenic transformation (0-50 cm agrogenic layer)

Soil	Depth, cm	Porosity, %					
		Total	Occupied with water	Occupied by physically stably bound water	Occupied by physically weakly bound water	Occupied by capillary water	Aeration
Typical chernozem with moderate humus content (Dendrarium 63 years)	0-10	63.8	31.9	8.1	4.6	19.1	31.9
	10-20	62.9	33.2	8.0	4.8	20.4	29.7
	20-30	62.5	33.6	7.8	4.7	21.3	28.5
	30-40	62.0	31.5	7.8	4.7	19.0	30.5
	40-50	31.8	30.8	7.8	4.9	17.7	31.4
Typical chernozem with moderate humus content (fallow ground 26 years)	0-10	65.2	33.2	7.3	4.4	21.5	32.0
	10-20	61.5	32.7	8.2	5.1	19.4	28.8
	20-30	59.7	32.5	8.4	5.2	18.9	27.2
	30-40	57.0	31.9	8.6	5.0	18.3	25.1
	40-50	53.2	29.0	8.6	5.0	15.4	24.2
Typical chernozem with moderate humus content arable	0-10	57.8	29.4	7.7	4.7	17.0	28.4
	10-20	56.1	29.2	8.1	5.0	16.1	26.9
	20-30	54.6	28.8	8.4	5.0	15.3	25.8
	30-40	53.4	28.5	8.4	5.3	14.8	24.9
	40-50	52.7	27.7	8.4	5.6	13.7	25.0

From Table 2, we can see that the aggregates in the sub-arable layer are characterized by the compaction of the aggregates and the reduction of the aggregate porosity. This allows us to conclude that the evolution of the compactness regime of the sub-arable layer of the arable chernozems is caused by a new process - luvic, that is not characteristic for the native chernozems, manifested in migration of the organo-mineral clay from the arable layer. This process is favoured by the more advanced level of leaching of the arable layer of the arable chernozems, the reduction of the calcium content retained in the adsorptive complex, the narrowing of the Ca: Mg ratio, the reduction of the humus content and the increase of its degree of mobility, the reduction of the degree of bond stability coagulation within organo-mineral compounds, increasing the degree of hydrophilicity of mineral colloids (Medvedev, 2014). All these contribute to the mobilization of the finely-dispersed fraction, its reorientation and periodic downward migration from the Aar/A horizon to Am-Bm and cause the unidirectional clogging of the aggregate pores.

The reduction of the aggregate pore volume causes the accumulation inside the aggregates of the products of incomplete decomposition of organic matter, the reduction of microbiological activity, the realization of reduction reactions in some isolated spaces uncharacteristic of the derno-chernozemic pedogenesis. More frequently, they are carried out in the spring or during periods of heavy rainfall when the humidity level corresponds to the values of the field capacity for water.

V. V. Medvedev admits that these reactions can also take place in other periods but in the spaces inside the aggregates. In the conditions when the porosity of the aggregates presents values below 40%, the mineralization processes of the organic matter and the transformational activity are significantly reduced. According to the quoted author, the derno-chernozemic process is located on the surface of the aggregates where they contact the interaggregate pores (Medvedev, 2016). The cited author believes that the derno-chernozemic process is no longer characteristic of all aggregates. In some of these, another process of decomposition-transformation of organic matter is probably carried out. It is certain that this is not done in the aggregates in the arable layer that is periodically subjected to work. On the other hand, in the 30-60 cm (overcompacted) layer, a series of features involved by them can be seen. Thus, the 30-60 cm layer turns out to be a metamorphosed formation (modified *in situ*) - a new genetic horizon as a result of the accumulation in it of the multi-year effects induced by overcompaction.

The reduced flow of water inside the aggregates, the increased aggregate density, the establishment of unfavorable conditions for the realization of the process of humus formation, all these indicate the reduction of the intensity of the chernozemic pedogenetic process (Table 4).

Table 4. Pedofunctional-genetic effects caused by the degradation of the settlement indices of arable chernozems in the Pridanubian area

Criteria	The content and direction of pedofunctional processes	Pedofunctional-genetic effects
Humification-mineralization.	The accumulation of partially decomposed and pseudohumic organic substances in the aggregate pores.	Reducing the intensity of typogenetic processes (formation and accumulation of humus; aggregation-structuring).
Biological and microbiological activity.	Reduction and degradation of biodiversity and biological activity of soils.	Partial (mosaic) abiotization of the subaerial layer.
Change of relations: solid phase-solid phase	Increasing the volume of physically (thermodynamically) bound water.	Conservation of substances in aggregates, reduction of their migration capacity in the soil profile.
The formation of liquid and solid surface leaks.	Spatial differentiation of substances within the landscape.	Intensification of erosion.
Pollution and overwetting of pressure elements of the landscape.	Migration of pollutants.	Accumulation of pollutants and reduction of biological processes.
Changing the water balance in the soil and the water regime.	Neohydromorphization of the soil.	Hydrometamorphism. Hydrological degradation.

The mentioned points to early features of a new type of migration and transformation of finely dispersed fractions in the overcompacted layer of arable chernozems.

In the underlying horizons, the difference between the bulk density values in native and arable chernozems is reduced to a minimum. This implies the conclusion that in native conditions the bulk density is a quasi-balanced parameter corresponding to bioclimatic conditions and in agrogenesis conditions it is an unbalanced parameter (Medvedev, 2016).

The phenomenon of overcompaction of arable chernozems has a residual-cumulative character which is caused by the reduction of the decompaction capacity as the degree of structure degradation increases as a result of the condensation of the structural elements. As the degree of degradation of the structure increases, the balanced values of the bulk density are established at a higher level (Сорочкин, 1989). In this context, an important factor favouring the overcompaction of arable chernozems is the humus loss that causes the reduction of aggregate stability.

Starting from this, we consider that the bulk density and the structural-aggregate state are interdetermined and interdependent. The structure of the soil is achieved by the dynamics of the degree of compactness and the last by the structural-aggregate state. The integrative index of these interactions is the volume, structure and dynamics of the porous space. Through this prism of ideas, the basic function of the soil structure is to ensure an optimal degree of compactness of the soil and an optimal settlement state for achieving eco- and agro-ecosystem functions. The way of structural-functional organization of the soil mass determines the structural-aggregate state expressed in the dimensions, properties of aggregates (aggregate density, aggregate porosity, aggregate stability) and the ratio between aggregates with various pedofunctional functions (Jigau, 2009). These, in turn, are a function of the composition and properties of the structural elements at the microaggregate level and their interaction characterized by the type of structural links.

From the perspective of the basic function of the aggregate structure of soils, the basic criterion for evaluating the quality of the structure is the dynamics of the degree of compactness of the soil (Сорочкин, 1989). Optimal quality of the structure is considered in the absence of seasonal pulsations of the structural-aggregate composition and the bulk density of the soil. This is considered satisfactory in the case of attenuated pulsations of the structural composition and bulk density. Unsatisfactory quality is characterized by a very high degree of seasonal variability of structural composition and bulk density.

Table 5. Dynamics of the structural-aggregate indices of the arable chernozems in the space between the Prut and Dniester during the vegetation period

Soil	Depth, cm	Sample collection period											
		At the beginning of the vegetation period						At the end of the growing season					
		The dimensions of the aggregates, mm. Content of aggregates, %											
		>10	5-10	5-1	10-0.25	<0.25	Ks	>10	5-10	5-1	10-0.25	<0.25	Ks
Argilic chernozem	0-25	15.10	33.42	44.73	82.76	2.14	4.80	22.79	7.97	39.25	66.80	10.47	2.00
	25-40	17.12	36.19	41.34	81.12	1.76	4.30	26.33	47.17	24.99	72.97	0.70	2.70
	40-60	9.70	27.27	52.84	88.83	1.67	7.91	22.35	40.29	33.53	76.54	1.13	3.26
Argilic chernozem	0-25	-	24.38	63.80	97.36	2.64	36.50	43.16	29.03	26.87	55.02	1.82	1.44
	25-40	12.40	35.00	48.90	86.19	1.41	4.53	38.91	35.75	23.95	60.48	0.61	1.53
	40-60	6.42	26.75	62.34	92.26	1.32	11.92	10.51	19.19	61.65	87.30	2.19	6.87
Leached chernozem	0-25	11.35	39.33	41.96	86.68	1.97	6.50	24.43	33.17	34.45	73.05	2.52	2.71
	25-40	30.82	25.38	37.49	67.39	1.79	2.07	18.66	38.16	31.56	80.57	0.77	4.14
	40-60	6.24	30.40	52.42	90.60	3.16	9.63	47.03	18.38	14.69	52.31	0.66	1.10
Typical chernozem with moderate humus content	0-25	24.70	33.17	35.51	74.15	1.15	2.87	32.37	17.00	31.52	59.60	8.03	1.48
	25-40	39.68	24.28	33.24	59.94	0.38	1.50	20.70	36.93	37.70	77.63	1.67	3.47
	40-60	20.04	22.02	45.57	78.06	1.90	3.56	22.93	25.19	42.40	73.26	3.81	2.74
Typical chernozem with moderate humus content	0-25	8.36	26.29	56.26	90.02	1.62	7.82	7.84	25.96	51.95	87.30	4.86	6.87
	25-40	25.00	32.87	38.91	74.02	0.63	2.89	71.01	14.12	12.73	28.73	0.73	0.40
	40-60	20.48	20.34	50.33	77.95	3.28	3.28	38.12	19.31	32.31	58.31	3.57	1.40
Typical chernozem with low humus content	0-25	18.82	19.39	37.09	74.66	6.52	2.95	7.40	21.18	47.23	84.02	8.52	5.26
	25-40	16.33	43.09	47.46	82.94	0.73	4.86	10.36	40.76	36.78	88.78	0.86	7.91
	40-60	15.15	40.33	50.15	84.56	2.29	5.48	1.30	23.52	55.99	85.17	1.53	5.74
Typical chernozem with low humus content	0-25	-	13.43	59.18	91.01	8.99	10.13	10.31	18.20	47.54	81.60	8.08	4.44
	25-40	10.36	40.61	43.26	87.14	1.50	6.78	12.67	30.87	50.55	85.55	1.78	5.92
	40-60	5.45	38.67	50.59	93.87	0.68	15.16	15.40	20.55	59.63	83.42	1.18	5.03

Table 6. The dynamics of the settlement indices of the arable chernozems in the area between the Prut and the Dniester

Soil	Depth, cm	Sample collection period			
		At the beginning of the vegetation period		At the end of the growing season	
		Indices of the state of settlement			
		Bulk density, g/cm^3	Total porosity, %	Bulk density, g/cm^3	Total porosity, %
Argilic chernozem	0-25	1.12	60.0	1.34	47.0
	25-40	1.28	50.2	1.37	46.7
	40-60	1.24	52.0	1.33	48.8
Argilic chernozem	0-25	1.08	57.5	1.30	48.8
	25-40	1.23	52.1	1.39	45.9
	40-60	1.20	53.8	1.36	47.7
Leached chernozem	0-25	1.03	59.4	1.32	48.0
	25-40	1.20	53.3	1.34	47.9
	40-60	1.16	55.4	1.30	50.0
Typical chernozem with moderate humus content	0-25	1.06	58.3	1.33	47.6
	25-40	1.24	51.8	1.39	45.9
	40-60	1.18	54.6	1.37	47.3
Typical chernozem with moderate humus content	0-25	1.10	56.6	1.34	47.2
	25-40	1.26	51.0	1.41	45.1
	40-60	1.20	53.8	1.37	47.3
Typical chernozem with low humus content	0-25	1.06	58.3	1.35	46.7
	25-40	1.28	50.2	1.46	43.2
	40-60	1.23	52.3	1.40	46.2
Typical chernozem with low humus content	0-25	1.02	59.8	1.29	49.2
	25-40	1.19	53.7	1.43	44.4
	40-60	1.17	55.0	1.37	47.3

Our research has highlighted an increased degree of instability during the vegetation period of the indices of structural-aggregate condition and bulk density (Tables 5, 6).

This allows us to conclude that in the Pridanubian area predominate arable chernozems with satisfactory quality of the aggregate structure, which implies the establishment, over time, of the phenomenon of settling and vertisolation.

Unlike compaction and overcompaction, settling of arable chernozems involves not only reducing the total volume of interaggregate pores but also reducing the volume and diameter of capillary aggregate pores and transferring them to the category of textures occupied by physically bound water.

This leads to the reduction of the swelling capacity of the aggregates and that of self-restoration and attributes to the compacted layer an unidirectional trend of exaggerated increase of soil mass per volume unit and the development of the vertisolation process.

According to И. В. Ковда the phenomenon of vertisolation involves 3 main notions: vertisolation potential, vertisolation, genesis of vertisolation (Ковда, 1995).

The vertisolation potential defines the degree of predisposition of the soil to vertisolation manifested in high values of hardness, volumetric mass, swelling, contraction.

The vertisolation potential depends on the chemical, mineralogical, granulometric composition, the content of organic matter, the waterstability of the structure. Some of the listed factors are inherited from the parent rock but transformed during the pedogenesis process. Another part (humus content, waterstability of the aggregate structure) is determined directly by the pedogenesis process (Сорокин, 2016). In this sense, we believe that it is more appropriate to distinguish between passive vertisolation potential (factors inherited from the parent rock) and active vertisolation potential (factors from pedogenesis).

Through this prism of ideas, the arable chernozems in the Pridanubian area have a high passive vertisolation potential determined by the increased content of fine clay and the predominance of minerals partially (illite-hydromic) and strongly (smectite-montmorillonite) swelling in the mineralogical composition of the fine clay (Table 7).

Table 7. Mineralogical composition of the fine clay fraction (‹0.001 mm) of weakly and moderately humiferous typical chernozems (Data provided by T. V. Popov)

Soil	Genetic horizon	Depth, mm	Fine clay content, %	Mineral content % of clay mass		
				Smectite montmorillonite	Hydromics	Kaolinite Chlorite
Typical clayey loamy arable chernozem with low humus content (Orhei district)	Aar 1	0-20	29	37	52	11
	Aar 2	20-35	31	43	48	9
	Am Bca	40-60	30	49	37	14
	Bca	60-75	28	49	39	12
Typical loamy arable chernozem with low humus content (Orhei district)	Aar 1	0-20	26	31	57	12
	Aar 2	20-35	24	35	55	10
	Am	35-45	24	35	54	11
	Am Bca	50-60	23	31	56	13
	Bca	70-85	21	30	60	10
Typical irrigated arable chernozem with moderate humus content (Orhei district)	Aar 1	0-20	29	51	44	5
	Aar 2	25-35	32	36	54	10
	Am	35-45	32	41	52	7
	Am Bm	50-65	31	39	49	12
	Bca	70-85	29	33	54	13

Realization of the vertisolation potential of chernozems, along with the listed factors, requires contrasting hydrothermal regime conditions. In this sense, the intensity of the vertisolation process is determined by the amount of atmospheric precipitation, temperature, and the dynamics of their change.

The wetting-drying regime, in the context of realizing the vertisolation potential, also depends on the relief conditions, the degree of natural drainage, the density of the vegetation cover, the presence and level of phreatic and pedophreatic waters.

Through this prism of ideas, the realization of the vertisolation potential of the chernozems in the Pridanubian space is favored by the alternation of states of excessive wetting, during periods with abundant precipitation, and those of rapid overdrying caused by high temperatures. The longer the period between precipitations, the greater the degree of vertisolation (Jigau, 2009; Fala and Jigau, 2018).

The decisive role in limiting the realization of the vertisolation potential of the chernozems in the region belongs to the process of formation and accumulation of humus and that of aggregation-structuring. In this sense, it was established that the more intensively they occur, the lower the degree of realization of the vertisolation potential. As a result, within the native chernozemic pedogenesis, the soil vertisolation process is only evidenced in the spaces where it takes place with the participation of phreatic waters, so that in the pedological investigations carried out in the 50s of the 20th century, the vertisolation of chernozems is evidenced in restricted spaces. This has increased in arable chernozems more recently as a result of the humus and soil structure loss under conditions of natural-anthropic chernozem pedogenesis (Jigau and Lesanu, 2012).

In the opinion of the cited authors, in the arable chernozems of the Pridanubian area, vertisolation is favored by the changes in the pedofunctional regimes and elementary pedogenetic processes induced by the intercalated action of agrogenesis and climate changes. At the same time V.S. Tshovrebov et al. believes that increasing of vertisolation potential and its realization in arable chernozems are favored by the processes of biological alteration of hydromics in the arable layer with the formation of smectite-montmorillonite and mobile colloidal compounds of silicium (Tshovrebov, 2017). According to the cited authors, the content of mobile colloidal silicon compounds in the arable layer of the chernozems exceeds their content in the A horizon of the native chernozems by 2-3 times, given that the hydromic alteration process does not take place in them because they have a balanced balance of potassium. In this context, they consider that the vertisolation of chernozems implies seasonally variable changes coming from the consolidation-deconsolidation of the soil mass, and its trend is determined by the direction and intensity of the transformation processes of the mineral component and has an unidirectional character.

Through the thermodynamic concept of the physical state of soils A.V. Smaghin believes that vertisolation is caused by the reduction (about 2 times) of the effective thickness of the water layer which is thermodynamically bound of the dispersed system with the intensification of the interactions between elementary soil particles (compaction effect) (Смагин, 2020).

According to the cited author, the vertisolation process is based on the physical-mechanical mechanisms of interaction of finely dispersed particles and water molecules within the swelling-contraction cycles.

In this context, vertisolation is a process of destruction of the initial structure of the soils that leads to the formation of vertic horizons with a massive blocky structure with a plastic consistency in the wet state and consolidated in the dry state. Through this prism of ideas, vertisolation is a secondary degradative process of a natural-anthropic nature that leads to the formation of metastructured arable and subarable horizons. Vertisolation is manifested in the consolidation of the soil mass from the agrogenic layer in blocks with a diameter > 50 cm, separated by deep cracks with a width > 3-5 cm. Thus, vertisolation represents the primary phase of the vertisolation process, which involves the evolution of the anthropo-natural chernozemic pedogenesis with the formation of secondary slitozems.

According to I.V. Kovda et al. genesis of vertisolation should be examined along with other types of pedogenesis: chernozemic, podzolic, halomorphic, etc. (Kovda, 1992).

In contrast to these, genesis of vertisolation is distinguished by cyclical self-development, which is manifested in the intensification or attenuation of the degree of complexity of the soil cover of agrolandscapes determined by the degree of interdetermined and interdependent physical degradation caused by the disruption of the functioning of the pedofunctional system [bioenergetic system ↔ aggregate system].

According to several authors, genesis of vertisolation includes a series of elementary processes corresponding to bioclimatic conditions. In the case of arable chernozems, genesis of vertisolation involves the processes of formation and accumulation of humus, transformation of clay minerals, argillization, metaaggregation-metastructuring (Jigau, 2021; Jigau et al. 2022).

In this context, depending on the concrete bioclimatic conditions, genesis of vertisolation assumes several distinct forms.

According to V.R. Volubuev and F.I. Kozlovski vertisolation of arable chernozems is caused by the increase in colloidal activity as a result of the partial decalcification of the agrogenic layer (Володуев, 1948; Козловский, 1987), the increase of magnesia content in the adsorptive soil complex as a result of the decalcification of the agrogenic layer, the increase of the dispersion factor of the agrogenic layer caused by the humus loss with the intensification of the degree of hydrophilicity of the fine clay (Jigau, 2021).

An important factor favoring the genesis of vertisolation of arable chernozems are the changes induced by agrogenesis in the composition and properties of the basic soil material.

Loss of biota and humus led to the significant unsaturation of the physical clay fraction (< 0.01 mm) with humus and the increase in the degree of colloidal activity.

At the same time, the unidirectional expropriation of potassium (K_2O) with crops from the agrogenic layer intensified the transformation processes of hydromics into smectite-montmorillonite.

According to some authors, in these conditions, under the action of the pressures from the swelling of the soil mass upon wetting, the coagulation bonds characteristic of the chernozemic structure are replaced by condensation bonds with subsequent irreversible consolidation and the increase in their degree of stability during the drying shrinkage process (Корнблюм, 1972).

In accordance with the above, the vertisolation of arable chernozems involves three genetic-evolutionary stages: a) transformational - which involves all the processes, interacted and interdetermined, of quantitative and qualitative changes in the basic soil material and the structural-functional organization at all hierarchical levels of structural-functional organization of the soil; b) consolidation - all the processes of replacing the coagulation bonds between the solid components with condensation bonds that lead directly to the formation of the vertisolation state; c) the

development and stabilization of the properties that determine the special vertisolation state due to the newly formed bonds as a result of the compaction and modification of the superficial properties (surface energy) (Корнблюм, 1977).
The bioclimatic and pedofunctional framework of the region creates conditions for the realization of the vertisolation process as a result of the modification of the structural links in the aggregate formations under the action of the pressures originating from swelling during wetting and contraction during drying.
The seasonal superficial overwetting, which has increased in the last 40-50 years, in the chernozemic areas, involved a new mechanism of vertisolation of the arable chernozems, which involves 2 phases: a) prevertisolation, which involves multiple processes of transformation of the basic matter of the soils in conditions of overwetting (quasi-gleyization, gleyization, montmorillonitization, argillization, solonetzization, etc.) materialized in increasing its vertisolation potential; b) vertisolation - the structural-functional organization of the soils in the newly created conditions with the formation of massive compacted blocks which leads to the extension of the vertisolation process upwards in the soil profile as a result of the residual-cumulative overwetting of the lower segment of the profile.
The vertisolation process in the lower layers of the profile is favoured by the mechanical barrier effects induced by the vertic layer on the soil surface.
In such conditions, through the preferential descended paths of water migration in the soil profile, water penetrates into the lower layers, and in dry periods the vertic layer on the surface creates impediments for capillary ascent and its physical evaporation from the soil surface.
The unidirectional vertisolation of the middle and lower segment is also favored by the lower waterstability of the aggregate structure as a result of the reduced content of humus and the greater weight of the aggregates formed with the participation of calcium carbonate. At the same time, in conditions of excessive humidity in the middle and lower layers of the profile, the activity of the mesofauna in the soil is significantly reduced (Fala, 2018). Depending on the evolutionary phase of the genetic-evolutionary chain, the degree of yield reduction, as a result of the change in the state of settlement, ranges from 5-25% depending on the crop in conditions of compaction-overcompaction, and up to 50-60% in conditions of settling/vertisolation (Fala and Jigau, 2018).
According to the same research the agro-technical processes practiced in order to optimize the agrogenic layer settlement indices, including deep loosening, only ensure its partial decompaction for a short period of time. Depending on the initial condition of the soils, the unfavorable settlement condition is restored in 1-2 years. At the same time, agrotechnical processes do not contribute to the decompaction and renewal of compacted structural aggregates. As a result, the effects caused by compaction persist even when the bulk density values fall within the range of optimal values. More than that, the residual compaction effects of the aggregates are maintained even under conditions of sustainable land management technologies (No-till, Mini-till). This implies the need to change the management paradigm of the agrogenic layer settlement indices in the framework of some bioremedial technologies based on the intensification of the biological processes of structural decompaction and stabilization of aggregates (Fala, 2018; Jigau, 2021). Summarizing the mentioned, we consider that the vertisolation of arable chernozems is a component part of the desertification process of chernozems (Figure 1).

CONCLUSIONS

The evolution of arable chernozems within the evolutionary-genetic chain "compaction – overcompaction - settling - genesis of vertisolation" assumes two hierarchical levels: a) the more rigid placement of the solid components materialized in the change in the ratio between the mass and the volume of the soil phases manifested in the total volume of the pores, its dynamics as well as the composition of the porous space: b) modification of the mechanisms and links of the formation of aggregates with the modification of their types materialized in the increase of aggregate density and the significant reduction of aggregate porosity.
This leads to the radical one-way modification of the pedological (hydric, thermal, aeration)

and pedofunctional (aerohydric, hydrothermal, redox) regimes with the involvement of aridization → desertification elements of the soil cover. Within the current climate trend in the region, the specified phenomenon is intensifying and increasing, being favored by the interdependent and interdetermined degradation of the pedofunctional system [humic system ↔ aggregate system].

Combating the specified degradative processes implies the need for a new management paradigm of the physical state of soils based on the sustainability of the functionality of the pedofunctional system [humic system ↔ aggregate system] within adaptive-landscape-bioremedial technologies.

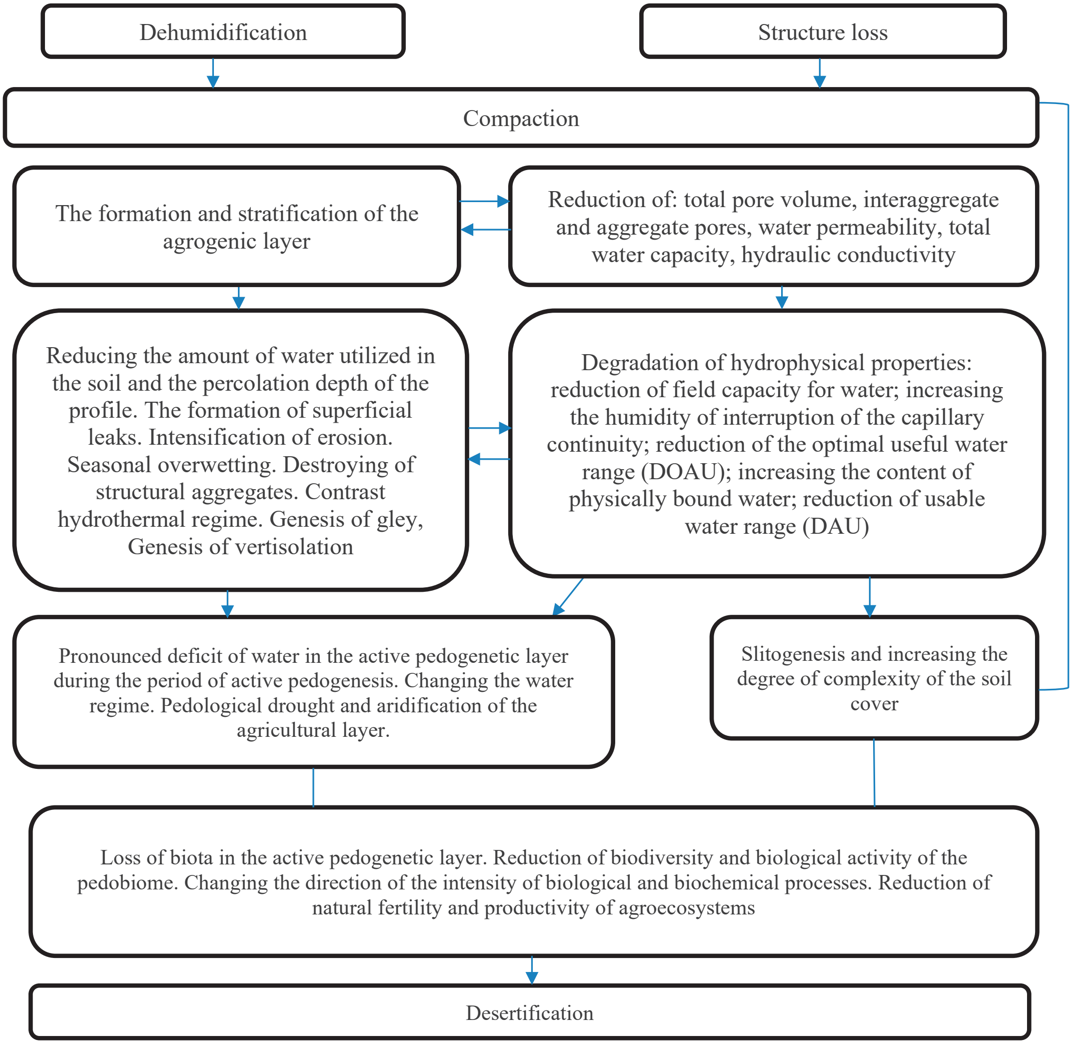

Figure 1. Hierarchy of desertification processes of steppe agro-landscapes under conditions of agrogenic modification of the state of settlement of arable chernozems

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LEACHING OF THE BASIC NUTRIENTS FROM IRRIGATED SOILS OF THE DRY SUBTROPICAL ZONE OF AZERBAIJAN

Tamara LEAH[1], Goshgar MAMMADOV[2], Turan MAMMADOV[3]

[1]Institute of Soil Science, Agrochemistry and Soil Protection "Nicolae Dimo", 100 Ialoveni Street, Chisinau, 2070, Republic of Moldova
[2]Institute of Soil Science and Agrochemistry, Ministry of Science and Education, M. Rahim 5, Baku, 1073, Republic of Azerbaijan
[3]Bursa Uludag University, Gorukle, Campus 16059 Nilufer, Türkiye

Corresponding author email: tamaraleah09@gmail.com

Abstract

The article presents the results of researches on the influence of water consumption and the length of the irrigation furrow on the leaching of nutrients and humus from irrigated soils of dry subtropics of Azerbaijan under tomato cultivation. The effect of different fertilization systems on tomato yields was also investigated. With a slope in a 100 - meter furrow at a water flow rate of 3.0 l/s, the content of leachable biogenic elements (N, P) and humus with soil was 0.03% for nitrogen, 0.02% for phosphorus and for humus 0.07%. Increasing water flow from 3.0 l/s up to 3.5 l/s negatively affects the leaching of humus and nutrients from the soil. With an increase in water consumption to 3.5 l/s, these indicators increased markedly by 0.01% for nitrogen and phosphorus, and by 0.02% for humus. It was found that with an increase in the length of the furrow to 200 meters, at the same norms, the flow of water, leaching of humus and nutrients was not observed. Using of an organic-mineral fertilizer system had a positive effect on the yield of tomato. So, in the free-fertilized variant, the tomato yield was 0.32 t/ha per at 100 m furrow.

Key words: *leaching of the basic nutrients, irrigated soils, fertilizers system.*

INTRODUCTION

The problem of protection, restoration and increase of soils fertility is relevant for arid and dry subtropical zones of many countries, including Azerbaijan. In particular, it is a great importance for irrigated lands. The area of irrigated land in Azerbaijan is 1.42 million hectares and more than 65% of these lands are located in areas with a significant slope. In the irrigated soils of dry subtropics, irrigation erosion processes develop, causing damage to agricultural production (Гурбанов, 2010; Гурбанов & Мамедов, 2009; Мамедов, 1989; Агрофизические..., 1966; Azizi et al., 2008).
Losses of nutrients from the soil and fertilizers are associated primarily with the amount of water entering in the soil and with the water regime of the soil. The research of the amount and composition of solid runoff carried out by waste water during irrigation is important for studying changes in the properties of irrigated soils (Доспехов, 1985; Мамедов, 1997; Лапа & Босак, 2006).
Using the fertilizers in irrigated eroded sloping soils has recently received little attention. In this regard, researches aimed at studying removing of nutrients with solid and liquid runoff during norms of irrigation, ratios and fertilizers, their dependence on the slope degree of the terrain are relevant (Заславский, 1983; Кудеяров & Семенов, 2004; Khomami et al., 2016).

MATERIALS AND METHODS

The object of the research was alluvial meadow-forest soils under irrigated conditions in the Guba-Khachmaz zone of Azerbaijan. The researches were carried out on the territory with a slope of 0.009 $tg\alpha$ under tomato cultivation. The experiments were laid in the Gusarchay experimental station of the Ministry of Agriculture of Azerbaijan located on the territory of the Khachmaz region. The laying of field experiments and carrying out phenological observations were carried out according to the method of field experiment Dospekhov B.A. (Доспехов, 1985; Кауричев & Лыков, 1979; Агрохимические методы..., 1975; Гусейнов, 1976). The degree of soil susceptibility to erosion and leaching of nutrients was determined according to Zaslavsky M.И. (Заславский, 1983; Лапа & Босак, 2006). Agrotechnical measures were carried out in accordance with agricultural rules adopted for the region.

Ammonium nitrate, simple superphosphate and potassium chloride were used as mineral fertilizers. The organic fertilizer was cattle manure at a moisture content of 60-65%, containing an average of 0.5% N, 0.25% P_2O_5 and 0.6% K_2O.

Annual norms of organic fertilizers were applied for ploughing in autumn (100%). Mineral fertilizers under tomato plants were applied at 3 terms during the growing season: when seedlings were transferred to the field; at the beginning of budding; at the start of fruiting.

Field experiments under irrigation conditions on alluvial meadow-forest soils under tomato culture were carried out in triplicate. To establish the leaching of nutrients with the washed off soil, the experiments were carried out according to the scheme indicated below (Table 1, Figure 1). Each plot is 200 m^2 (tomato planting pattern 70 x 30 cm), in each variant - 95 plants. In the experiments, the furrow length - 200; 150; 100 m and water flow - 3.0 and 3.5 l/s.

Table 1. Conditions and scheme of the experience

Soil	Slope, *Tgα*	Water consumption, l/s	Furrow and strip length, m
Furrow irrigation, tomato culture			
Alluvial meadow-forest	0.009	3.0; 3.5	100, 150, 200

On the experimental site, the slope of the relief was 0.009 *tgα*. The furrow length is set in three options, i.e., 100, 150 and 200 m. In each variant, different water flow rates were taken along the furrows, 3.0 m/s and 3.5 m/s.

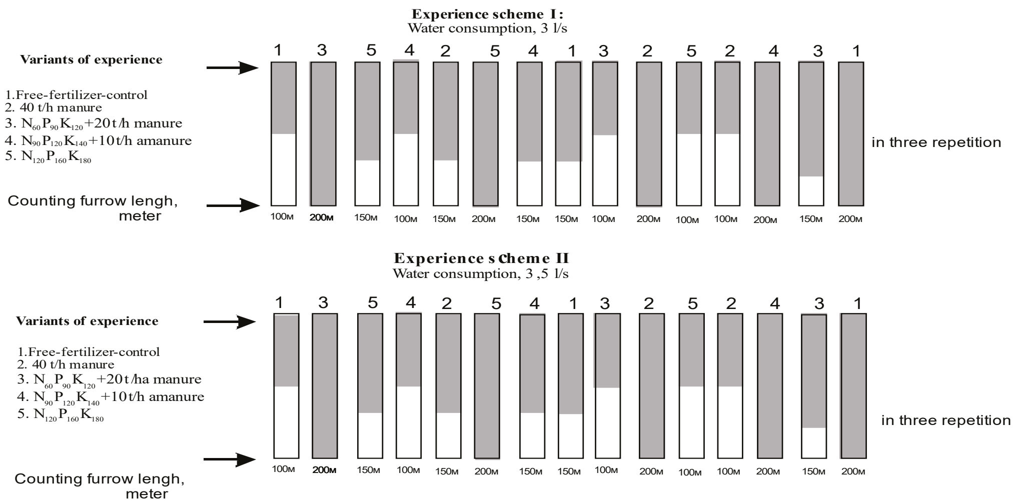

Figure 1. Experience scheme I and II

RESULTS AND DISCUSSIONS

As a result of many years of field experiments, it has been established that the application of mineral fertilizers for vegetable crops on sloping lands, during irrigation, should be differentiated, taking into account the elements of the slope and irrigation soil erosion (Мамедов, 1997, 2010, 2017; Arncon et al., 2005; Гусейнов, 1976); Мовсумов, 1978).

The soils of the Guba-Khachmaz zone of the north-western part of the Greater Caucasus within Azerbaijan are intensively used in agricultural production. Some of these soils are subject to the process of irrigation erosion, that is, leaching of the main nutrients and humus with the soil.

To study the agrochemical characteristics of the soils of the experimental plots, soil sections were laid and soil samples were taken along the genetic horizons, in which soil properties and their significance for fertility (Гурбанов, 2010; Мамедов, 1997; Edwards et al., 1996; Arncon et al., 2005) were studied according to generally accepted methods.

In experimental plots with vegetable crops under irrigated conditions (Table 2), the agrochemical properties of alluvial meadow-forest soils were carried out and studied.

Table 2. Agrochemical properties of irrigated soils in dry subtropics Guba-Khachmaz zone of Azerbaijan

Alluvial meadow-forest soils														
Genetic horizons	Depth, cm	Common humus, %	Common nitrogen, %	$N\text{-}NH_4$			P_2O_5			Common, %	K_2O		pH water
				water soluble	absorbed	NO_3, %	Common, %	water soluble	mobile		water soluble	exchangeable	
				mq/kq				mq/kq			mq/kq		
A_p	0-18	3.95	0.24	4.95	17.4	8.13	0.16	4.2	23.2	3.66	25.4	231	7.5
A_1	18-37	2.87	0.17	3.52	11.2	5.25	0.12	3.1	21.7	3.18	24.7	208	7.8
B_1	37-65	1.75	0.11	3.05	9.75	3.10	0.09	2.2	13.1	2.56	21.5	198	8.1
B_2	65-90	1.06	0.04	2.16	6.84	1.08	0.06	1.5	10.2	1.73	17.8	160	8.0
C	90-115	0.41	0.01	1.12	4.92	0.12	0.03	1.2	7.2	1.43	14.7	139	8.1

In the arable soil layers, the humus amounted 3.95%; total nitrogen - 0.24%, and absorbed ammonium - 17.4 mg/kg of soil. Mobile phosphorus (P_2O_5) in the arable layer was 23.2 mg/kg, and total phosphorus - 0.16%; in the arable soil layer, exchangeable potassium - 231 mg/kg of soil, while total potassium was 3.66%. Subsurface soil layers were also studied, genetically soil horizons up to 115 cm. These indicators gradually decreased, respectively, in terms of gross value, humus to 0.41%; nitrogen (N) - up to 0.01%; phosphorus (P_2O_5) up to 0.03% and potassium up to 1.43%. With increasing depth, the content of humus and basic nutrients gradually decreased in the genetic horizons. Thus, alluvial meadow-forest soils are poorly provided in terms of the content of basic nutrients and humus content.

We also studied the structural composition (water-stable aggregates) of the soils of the experimental plot (Table 3). The structure of soils is closely related to their genesis; depending on the soil and climatic conditions, a certain shape and water resistance of soil aggregates are formed. Maintaining favorable physical conditions in the soil necessary for a particular crop is facilitated by the presence of an agronomically valuable water-resistant of fine-grained structure in its (Мамедов, 2017; Khomami et al., 2016; Mammadov & Leah, 2020, 2021; Boincean & Dent, 2019).

Numerous studies indicate that soil structure changes the direction of physico-chemical and biological processes, and also affects the nature of plant growth and development and crop quality (Leah, 2010; Leah & Ceban, 2019).

Structural soils are characterized by favorable physical properties and a good nutritional regime, water and air easily penetrate into them.

Table 3. Structural composition (water-stable aggregates) of soils in dry subtropics of the Guba-Khachmaz zone of Azerbaijan

Alluvial meadow-forest soils (under vegetables), %					
Genetic horizons	Depth, cm	Aggregate size, mm			
		<0.25	0.25-1.0	1.0-10.0	Total >0.25
A_p	0-18	79.6	16.2	4.2	20.4
A_1	18-37	76.3	14.4	9.3	23.7
B_1	37-65	28.2	19.3	52.5	71.8
B_2	65-90	30.8	30.4	38.8	69.2
C	90-115	34.1	29.0	36.9	65.9

As can be seen from the data in the Table 3, water-stable aggregates in the arable layer in the amount of 0.25-1.0 mm were also 16.2% which indicates a poor soil structure.

The content of humus, gross nitrogen and phosphorus was determined in the solid runoff (Table 4).

Table 4. The content of gross humus, gross forms of nitrogen and phosphorus in solid waste, % (in the control version)

Slope, *tgα*	Water consumption, l/s	Furrow start			Furrow end (waste water)		
		Humus	N	P	Humus	N	P
	At 100 m furrow						
0.009	3.0	3.86	0.22	0.17	3.81	0.19	0.15
	3.5	3.86	0.22	0.17	3.79	0.17	0.13
	At 150 m furrow						
	3.0	3.86	0.22	0.17	3.84	0.21	0.16
	3.5	3.86	0.22	0.17	3.81	0.19	0.15
	At 200 m furrow						
	3.0	3.86	0.22	0.17	3.90	0.24	0.18
	3.5	3.86	0.22	0.17	3.92	0.25	0.19

Note: 1^0 slopes=0.017 tga

The content of humus, nitrogen (N) and phosphorus (P) at the beginning of the furrow (100, 150 and 200 m) was 3.86%, respectively; 0.22 and 0.17%, when irrigating in furrows with a water flow rate of 3.0 l/s and 3.5 l/s.

At a water flow rate of 3.0 l/s and 3.5 l/s, the content of total forms of humus, nitrogen and phosphorus at the end of the furrow was 3.81%, respectively; 0.19 and 0.15% in the soil. At the end of the 150 m furrow, the content of total forms of humus, nitrogen and phosphorus gradually increased. At the end of the 200 m furrow, the content of total forms of these elements was at a water flow rate of 3.0 l/s, respectively - 3.90; 0.24; and 0.18%, and at a water flow rate of 3.5 l/s, respectively - 3.92; 0.25 and 0.19%.

On the field experimental plot, removing of biogenic elements and humus with washed out solid runoff from alluvial meadow-forest soils was also studied at water flow rates of 3.0 and 3.5 l/s (Table 5).

Table 5. Removing (Leaching) of biogenic elements and humus with washed soil during irrigation of agricultural crops in the control variant on experimental plots, %

Slope, $tg\alpha$	Variant		Humus	N	P
	Furrow length, m	Water consumption, l/s			
0.009	100	3.0	0.07	0.03	0.02
		3.5	0.09	0.04	0.03
	150	3.0	0.02	0.01	-
		3.5	0.04	0.02	0.01
	200	3.0	-	-	-
		3.5	-	-	-

The results of soil analyses for the content of humus, total nitrogen and phosphorus, as well as mobile forms of these elements, testified to a significant change in soil fertility, depending on the elements of the slope and leaching of humus, nutrients with the soil as a result of irrigation.

Irrigation waters of the irrigation systems of the Republic of Azerbaijan carry out an average of 0.66-0.98 kg of $N\text{-}NH_4$ and 0.69-0.88 kg of $N\text{-}NO_3$ per season; 2.7-7.0 kg P, 44-70 kg K and 84-162 kg of organic matter/ha.

The influence of different furrow lengths (m) and water consumption (l/s) in different irrigation rates has a different effect on the leaching of N, P and humus with washed away soil in the alluvial meadow-forest soil.

Thus, in 100 m furrow, at a water flow rate of 3.0 l/s, the content of leached biogenic elements (N, P) and humus with washed soil was 0.03% for nitrogen, 0.02% for phosphorus, and 0.07% for humus. With increased water consumption up to 3.5 l/s, these indicators increased by 0.01% for nitrogen and phosphorus, and by 0.002% for humus.

Leaching of humus and nutrients with the washed away soil was not observed with an increase in the length of the furrow to 200 meters, at the same water consumption rates.

When the furrow is extended up to 200 m, and the water flow rate is 3.0 l/sec the leaching of biogenic elements decreased and stopped in comparison with the 100 m furrow.

Leaching of the total forms of biogenic elements (N, P) and humus gradually stabilized with the lengthening of the furrow to 200 m at the same water discharge rates and was more effective than in a short (100 m) furrow.

It was found that at a water flow rate of 3.5 l/s, the leaching of biogenic elements and humus was higher than at a water flow rate of 3.0 l/s.

The amount of removal of nutrients and gross humus during furrow irrigation was directly dependent on the amount of water consumption.

We also studied the effect of the fertilizer application system when nutrients were washed out with washed soil on the yield of a tomato plant (Table 6).

Table 6. Tomato yield on alluvial meadow-forest soils under irrigation, depending on the removing of humus and biogenic elements N, P_2O_5 (water flow rate of 3 l/s)

Fertilizer system	Dose of fertilizer	Counting furrow length, m			Productivity, t/ha		
					average	Increase from option 1 (from control)	
		100	150	200		t/ha	%
Free-fertilizer-control	0	32.4	32.9	33.5	32.9	-	-
Organic	40 t/ha manure	49.5	49.7	50.1	49.7	16.8	51.1
Organic-mineral	$N_{60}P_{90}K_{120}$+20 t/ha manure	52.8	53.0	53.2	53.0	20.1	60.9
Organic-mineral	$N_{90}P_{120}K_{140}$+10 t/ha manure	47.9	48.7	49.0	48.5	15.6	47.3
Mineral	$N_{120}P_{160}K_{180}$	45.2	45.3	45.5	45.6	12.6	38.3

Fertilizer systems as well as various furrow lengths and irrigation rates had a different effect on the tomato yield. The average tomato yield (100, 150 and 200 m^3) was 32.9 t/ha in the experimental plot under vegetable crops in the control variant. In the experiment, positive indicators were noted in a 200-meter furrow at various water rates when using the organic-mineral fertilizer system at the rate of $N_{60}P_{90}K_{120}$ + 20 t/ha, and amounted to 54.2 t/ha. With a furrow length of 150 m and 100 m, the yield was, respectively, 53.4 and 52.8 t/ha, which indicates a positive factor for furrow lengthening up to 200 meters, with the same fertilizer application rates and at different water consumption rates (3.0 and 3.5 l/s).

With a water flow rate of 3.0 l/s, using of organic, organo-mineral at the rate of $N_{60}P_{90}R_{120}$ + 20 t/ha of manure and mineral fertilizer systems, the average tomato yield increased compared to the control, respectively, to 49.7, 53.0 and 45.6 t/ha.
When using the organo-mineral fertilizer system in the $N_{60}P_{90}K_{120}$ variant + 20 t/ha of manure, in a 200 m furrow, the yield was higher by 2.0 t/ha compared to 150 m furrow and by 1.4 t/ha compared to 100 meters.
Thus, using of different fertilizer systems and different furrow lengths had a different effect on the yield of tomato, under irrigation conditions on alluvial meadow-forest soils.
The organic-mineral fertilizer system had a positive effect on the yield of tomato. So, in an unfertilized version, on a 100 m furrow tomato yield was 32.4 t/ha. The yield gradually increased due to the use of fertilizers with the lengthening of the furrow to 150 and 200 meters, and a decrease in the leaching of nutrients. In these variants, the tomato yield reached 32.9-33.5 t/ha, respectively. The effect on increasing the yield of the same doses, with the lengthening of the furrow, is due to the leaching of nutrients from the upper runoff. Since when using the organic-mineral fertilizer system in the norm $N_{60}P_{90}K_{120}$ + 20 t/ha of manure, the tomato yield in 100, 150 and 200 m furrows, respectively, was 52.8; 53.0 and 53.2 t/ha. At the same time, it was found that, compared with a 100 m furrow in 150 and 200 m furrows, the tomato yield is gradually increasing.
In the fertilized variants, the regularity of the removing out of nutrients and humus with the washed away soil did not change, depending on the slope degree of the relief and the length of the furrow.
Thus, when using an organic-mineral fertilizer system in the norm of $N_{60}P_{90}K_{120}$ + 20 t/ha of manure (200-meter furrow in a water flow rate of 3.0 l/s), The average tomato yield was 53.0 t/ha, while the increase to the unfertilized variant was 20.1 t/ha or 60.9%. Table 7 shows the results of taking into account the yield, when irrigating with a water flow rate of 3.5 l/s.
With an increase in the irrigation rate and water consumption up to 3.5 l/s due to increased soil leaching of the main biogenic elements and humus, the yield gradually decreased depending on the applied fertilizers.
With a change in water consumption rates, that is, at a rate of 3.5 l/s, in the variant without fertilizers, the yield was 32.1 tons per hectare at a 100-meter furrow and when the furrow was extended to 200 meters, a pattern was also observed at the same water consumption rate. At high rates (3.5 l/s) of water flow, there was a constant decrease in tomato yield compared to lower rates (3.0 l/s).

Table 7. Tomato yield on alluvial meadow-forest soils under irrigation, and depending on the removing of humus and biogenic elements (N, P_2O_5) with washed away soil (at a water flow rate of 3.5 l/s)

Fertilizer system	Dose of fertilizer	Counting furrow length, m			Productivity, t/ha		
					average	Increase from option 1 (from control)	
		100	150	200		t/ha	%
Free-fertilizer-control	0	32.1	32.3	32.6	32.3	-	-
Organic	40 t/ha manure	49.2	49.3	49.6	49.4	17.0	52.6
Organic-mineral	$N_{60}P_{90}K_{120}$+20 t/ha manure	52.4	52.8	53.0	53.0	16.4	63.9
Organic-mineral	$N_{90}P_{120}K_{140}$+10 t/ha manure	47.8	48.2	48.5	48.2	15.8	48.9
Mineral	$N_{120}P_{160}K_{180}$	45.1	45.3	45.7	45.5	13.2	40.8

CONCLUSIONS

The content of biogenic elements and humus in the arable layer of meadow-forest soils was generally low. The content of humus, nitrogen and phosphorus was 3.95, 0.24 and 0.16%, respectively, with an increase in the depth of genetic horizons, these indicators gradually decreased.
Water-resistant aggregates in the arable soil layer in the section 0.25-1.00 mm (meso-structure) were 16.2%, which indicates a poor structural condition of the soil.
In the non-fertilized variant, the content of total forms of humus, nitrogen and phosphorus in the solid runoff (%) was determined in the control variant. So, at the beginning of the furrow, the content of total humus, nitrogen and phosphorus was 3.86, 0.22 and 0.17%, respectively, and the removing of biogenic elements and humus with washed soil under the tomato crop was established. Washout in a 100-meter furrow at a water flow rate of 3.0 l/s is 0.07% for humus, 0.03% for nitrogen and 0.02% for phosphorus. With the lengthening of the washout furrow, it gradually stabilized and no washout was observed in the 200 m furrow.

Increasing water flow from 3.0 l/s up to 3.5 l/s negatively affects the leaching of humus and nutrients from the soil. With a water flow of 3.0 l/s in a 100-meter furrow, the removal with washed soil for humus was 0.06%, for nitrogen 0.03%, and for phosphorus 0.02%. With an increase in water flow to 3.5 l/s, in the same furrow, the removal of humus and biogenic elements from washed soil increased by humus to 0.08%, by nitrogen to 0.04%, and by phosphorus to 0.03%.

The maximum losses of nutrients and humus were observed in the upper part of the slope in a 100-meter furrow at a water flow rate of 3.5 l/s. They gradually decreased with the lengthening of the furrow.

Study of the effect of mineral, organic and organic-mineral fertilizer systems on tomato yield, depending on the leaching of humus, nitrogen and phosphorus, at different furrow lengths and different water flow rates.

Best average yield across all furrows at water flow rate 3.0 l/s obtained by using organic-mineral fertilizer system in the norms $N_{60}P_{90}K_{120}$ + 20 t/ha - 53.0 t/ha. At the same time, the increase from the control amounted to 20.1 t/ha or 60.1%.

In a 200-meter-long furrow with a slope of 0.009 *tgα* and a water flow rate of 3.0 l/s, due to the least leaching of nutrients from the soil, the tomato yield increased compared to other options. With an increase in water consumption to 3.5 l/s with the same length of the furrow and with the same rate of fertilizer application, due to the large leaching of soils with irrigation runoff, a decrease in tomato yield was found.

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ECONOMIC EFFICIENCY OF SOILS FROM BANAT'S VINEYARDS

Casiana MIHUȚ, Anișoara DUMA COPCEA, Lucian NIȚĂ, Adalbert OKROS, Simona NIȚĂ, Daniel POPA, Vlad MIRCOV, Corina SÎRBU

University of Life Sciences "King Mihai I" from Timisoara, 119 Calea Aradului Street, Romania

Corresponding author email: casiana_mihut@usab-tm.ro

Abstract

This paper presents a qualitative evaluation of soils from the main vineyards of Banat, generally recognized by its red wines. Strictly viewed from a qualitative point of view, the soils cultivated with grapevines fall into the medium or low fertility category. However, if certain requirements are met - exposition, slope, soil land climate conditions – these soils can ensure the necessary conditions for cultivating grapevines and obtaining high and good quality productions. The geological conditions in which the soils of this area have been formed and evolved - in Moldova-Nouă, on shale, quartz and calcite; in Buziaș, on carbonate clays; and Recaș and Tyrol, on poorly reddish and slightly carbonated clays and loams.

Key words: *soil, vineyard, economic efficiency, fertility.*

INTRODUCTION

According to the latest data presented by Business Microcredit IFN SA, in 2022, Romania ranked sixth in the EU in grapevine-cultivated areas, with about 190,000 ha, after Spain (almost 950,000 ha), France (over 800,000 ha) and Italy (almost 650,000 ha) (Antoce, 2017).

Romania has 37 vineyards, of which are 123 viticultural centers, plus 40 independent viticultural centers located outside the vineyards. The main wine regions of our country are: Moldova, Muntenia and Oltenia, Transylvania, Banat, Crișana and Maramureș, and Dobrogea (https://businessmicrocredit.ro/romania-a-exportat-peste-18-milioane-litri-de-vin-in-2019).

Most soils in the vineyards are anthropic, poorly structured, with low humus content and reduced capillary porosity. On such lands, the appropriate execution and the optimal time for soil processing is very important (Dobrei et al., 2010; Contoman, 2011).

Literature (Fredrikson, 2011; Gaviglio, 2011) highlights the advantages and disadvantages of using the different soil maintenance systems in vineyards. Dobrei et al. (2005) studied, oer the years, the influence of soil work on local varieties in the grapevine-growing areas of Recaş, Miniş, and Buziaş.

Best locations for a vineyard are those that have well-structured soils with optimum fertility and moisture which provides a favorable environment for vines root system development (Ohmart et al., 2011).

For winegrape production, soil quality can be considered as the soil's ability to support the production of a crop while minimising negative effects on the environment. Although the concept of soil quality has been developed in several papers as an indicator of sustainable land management (Pankhurst et al. 1995; Doran and Zeiss 2000; van Bruggen and Semenov 2000), there is no accepted measure of soil quality available to Australian viticulture.

The evolution of world viticulture in the future will depend on the current organization and on the historical, economic, cultural, scientific, social and religious influences that differ from one country to another (Fredrikson, 2011; Okros, 2015).

The culture of grapevine in Romania is part of the future of the world grapevine-growing, but with particularities imposed by the socio-economic conjuncture, the objectives pursued in the next and later stages, meant to make an outline for the viticulture of the beginning of the third millennium.

Teodorescu shows that, from a climate point of view, in the wine regions of Romania, the years with suitability for the quality of the wines are characterized by the low amount of precipitation during the active vegetation

period, especially since August, correlated with the lower proportion of the temperature degrees and the hours of sun (Mircov, 2021).
These climatic characteristics are also found in the areas under study, thus explaining the constant high quality of the wines (Mihuț et al., 2018).
The soil is the material support on which the grapevines develop. Its fertility features, as well as the relief, the altitude, and the exposition, determine its variability (Țărău et al., 2007). Grapevine is a plant that covers the most varied types of soil and has a very good adaptation capacity.
The physical properties of the soil indirectly have a strong influence on the growth and fruiting of plants, as the water and air regime, as well as the microbial life and the transformation of the nutrients in the soil, depend on them (Constantinescu et al., citation Drăgănescu, 2002).

MATERIALS AND METHODS

The studies were carried out in the main viticultural centers and vineyards in Banat: Moldova-Noua, Buziaș, Recaș, and Tyrol.
After identifying the main types of soil, the pedoclimatic potential was scrutinised and the economic efficiency of these soils for the grapevines was established.
The soils are of the rendzine, eutricambosol, preluvosol, luvosol, antrosol, and regosol type, along with soil associations.
In order to reach the proposed objectives, research methods specific to the soil science field were used: identification an description of the soil type, expeditious determinations in the field, laboratory analyses, processing of soil information, etc. (Mihuț, 2018).

RESULTS AND DISCUSSIONS

Banat's vineyards are located in the southwest part of Romania on the territory of Timiș and Caraș Severin counties and include about 3,000 ha, with island-arranged plantations. These plantations are distributed in several viticultural centers, which have some uniformity of the soil an climate features and meet, to some extent, the conditions of a single vineyard. The climate is of sub-Mediterranean type, characterized by gentle winters, hot summers, and long autumns, which ensures a good maturation and even over-maturation of the grapes (Mihuț et al., 2018).
Rendzine soils (limestone soils), eutricambosols, preluvosols, luvosols, antrosols and regosols, along with soil associations, predominate. The main viticultural centers in this region are Moldova Nouă, Tyrol, Silagiu (Buziaș), Recaș, and Teremia. The viticultural centers of Moldova Nouă, Recaș, Buziaș, and Tyrol, whose area is 2,903 ha as shown in Figure 1 are not included in any grapevine region.

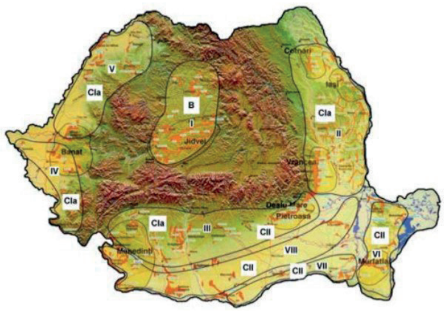

Figure 1. Classification of the grapevine regions in Romania

According to the data provided by the site www.timis.insse.ro, the western region of Romania comprises four counties (Arad, Caraș-Severin, Hunedoara, and Timiș), which, together, make up the Western region with 5,733 ha cultivated with grapevines: some of these vineyards are grafted and others are hybrid, as shown in Table 1.

Table 1. Area of fructifying vineyards in the Western region (ha) (after www.timis.insse.ro)

County	Fructifying vineyars		Gafted and indigenos vineyards		Hybrid vineyards	
	Total	Of which: mainly private property	Total	Of which: mainly private property	Total	Of which: mainly private property
Arad	2,524	2,524	2,104	2,104	420	420
Caraș-Severin	554	554	350	350	204	204
Hunedoara	11	5	6	-	5	5
Timiș	2,644	2,644	2,249	2,249	395	395
Total Western region	5,733	5,727	4,709	4,703	1,024	1,024

The largest areas occupied by fruit vines in this part of Romania (Western region) are occupied by the counties of Timiș, with 2,644 ha and Arad with 2,524 hectares, at the opposite pole is the county of Hunedoara, with only 11 ha and Caraș-Severin, with 554 ha. Of the total area, hybrid vines occupy very little, most of the vines are grafted and indigenous.
At the level of Timiș and Caraș-Severin counties, the situation is presented in Figure 2.

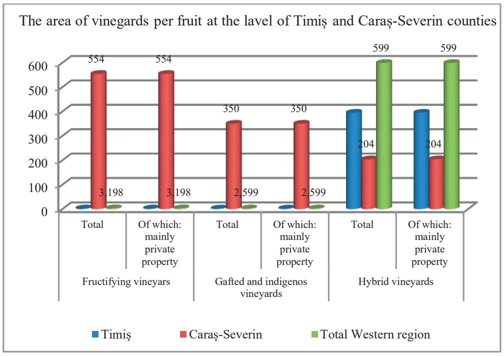

Figure 2. The area of vineyards per fruit at the level of Timiș and Caraș-Severin counties

The four vineyards and viticultural centers studied:

1. The Moldova-Nouă Viticultural Center occupies an area of 260 ha and is located in the hilly area of Banat, on slightly high hills of the Dognecea Mountains, at altitudes between 160-350 m; most of the grapevine plantations are located on plateaus with slopes of 9-20% and southern, south-western and southeastern exposition.
There, the following types of soils were identified: regosols, luvosols, and eutricambosols, the largest area (over 70%) being represented by regosols, which have a high content in skeleton (11.2-33.8), iron and microelements.
The soils in this viticultural center are very poorly supplied in humus and total nitrogen and very rich in total phosphorus and poor in potassium.
The water regime of the soil during the vegetation period differs from one type of soil to another, within wide limits, but does not affect the physiological functions of the grapevines. In dry years, however, there are certain varieties that suffer from the lack of precipitation. On luvosols, the Cadarcă variety has great vigour, the foliar area and the length of the annual shoots increase with over 25% than on eutricambosols and with 45-62% more on regosols. It has also been observed that the weight and efficiency of the Cadarcă variety is higher on eutricambosols compared to regosols, but on regosols there is better quality and higher (8-21%) sugar content.
In this vineyard, the eutricambosols located on the slope, which have a high percentage of skeleton and an average texture, contribute to high quality red wines.
Regosols are soils formed and evolved on non- or low-consolidated parental materials. The physical features of the regosols are favorable, and chemical oes are restrictive, with a low natural fertility. They suit grapevines well, being classified 5^{th} grade with 58 points, while luvosols have an average fertility, being classified in 6th grade with 49 points.

2. The Recaș Vineyard occupies an area of 1,589 ha; it is located mostly on slopes with southern exposition, at an altitude between 120-180 m (Figure 3). In this vineyard, three types of soil were identified: preluvosol, luvosol and eutricambosol.

Figure 3. Recaș Vineyard

Preluvosols were formed on clays and loams and occupy 30% of the total area of vineyard being characterized by the following features:
- The texture is medium (clayey-dusty and clayey) between 0-45 cm, and clayey-loamy in the horizons below 45 cm due to the increase of the illuviated loam content;
- The reaction of the soil is acid between 0-24 cm and low acidic between 24-115 cm;
- The humus content is 2.25% in the Ao

horizon and 2.13% in the transition horizon AB;
- The content in mobile phosphorus is medium;
- The content in the mobile potassium is small.

The suitability of this soil for grapevines is less favourable, it is classified 2nd with 40 points.

Luvosols occupy 40% of the territory; they are met on slopes of 10-12% and have the following features:
- The texture is clayey-dusty between 0-40 cm and clayey-loamy between 40-57 cm, with a textural differentiation index of 1.82;
- The reaction of the soil is medium acidic on the surface, strongly acidic (5.26) between 28-57 cm, and medium acidic (pH 6.60) below 58 cm;
- The content in humus is 2.09% in Ao and decreases to 1.40%;
- The content in phosphorus and mobile potassium is reduced.

Luvosols, although they have a low natural fertility for other crops, in terms of vines, this soil places them in class V with 53 points.

Eutricambosol occupies 30% of the area of the vineyard; it is a highly-levigated soil formed on carbonated clays and marls, it is found on slopes 20-30%, and it has the following features:
- The texture is clayey-dusty in the Ao and transitional AB horizons and deeper, in the Bv and BC horizons, it becomes clayey-medium-loamy;
- The reaction of the soil is low acidic (pH between 6.05 and 6.25);
- The content in humus is 2.43% between 0-22 cm and it reaches 1.37% between 22-41 cm;
- The content in mobile phosphorus is low;
- The content in mobile potassium is medium.

Regarding the suitability of this type of soil for grapevines, it falls into the group C or Suitable with 60 points.

These soils generally provide good conditions for cultivating and developing grapevines, with a porosity of 40-55% and a clay content between 14-35%. Groundwater is located at depths between 1.5-3 m and has a low chloride and phosphate content.

From a climatic point of view, the Recaș viticultural center is characterized by an average annual rainfall ranging from 650-800 mm, and by average annual temperatures between 7.2-10.4^{0}C. Annual aridity indices are between 35-45, and evapotranspiration is lower than the average rainfall. The water regimen is percolative, favouring debasification an the migration of the clay along the profile.

3. The Silagiu Viticultural Center (Buziaș) is located in the southeast of Timiș County in the Silagiului hills, at an altitude between 230-320 m. The soils identified there are represented by luvosols, preluvosols and anthrosols, predominantly being the antrosols followed by luvosols.

Luvosols have evolved on clayey and loam deposits; on the surface, they have a medium texture (clayey-sandy, dusty-clayey dusty-loamy), with a high percentage of coarse sand and, along the profile, medium-fine (clayey-lamy) and fine (clayey-sandy). Total porosity has values of 45-50%, optimal values for the culture of grapevines. The soil reaction is low acidic to neutral or low alkaline with values that fall within optimal limits for cultivating grapevines (5.5-8.5). Humus content is reduced (1-2%) and it corresponds to the soils suitable for the cultivation of grape varieties for high quality wines. Groundwater is found at a depth of 10 m.

The climate is a moderate temperate continental one, with Mediterranean and Oceanic influences. The springs are early, short and quite warm, but there were also low temperatures in April and May because of a cold air front from north and northwest of Europe. Annual rainfall is between 700-800 mm, with a multiannual average value of 655 mm. The highest amounts of rainfall are in June and May.

These soils are classified 2nd and 3rd quality for grapevines culture.

The Silagiu Viticultural Center has, as a main direction of production, high quality white wines (Italian, Sauvignon, and Muscat Ottonel varieties predominate). However, high quality red wines from the varieties are also produced: Cabernet Sauvignon, Pinot Noir, Merlot, and Burgund.

4. The Tirol Viticultural Center, is located in the south-western part of Romania (Caraș-Severin county) in the grapevine area of

Banat's Hills, near the village of Tirol, west of Bocșa. This viticultural center occupies an area of 285 ha, on the Tyrolean Hills at an altitude between 140-180 m, on slopes whose inclination reaches up to 15%. The total area cultivated with vines within the commune of Doclin, to which the village of Tiroul also belongs, respectively this wine-growing center, is 327 hectares.

This viticultural center is located in a hilly area, the soils identified are districambosols and preluvosols, dominant in a proportion of over 80% are districambosols formed on clays. Districambosols are acidic soils, they have a pH between 5.6 and 5.8, a low humus content, with values between 1.03 and 1.88% at the depth of 0-20 cm, respectively between 0.55 and 0.75% at the depth 20-40 cm. They are poorly supplied in total nitrogen and assimilable potassium and moderately supplied in phosphorus. The soils that are located on the plateaus are very poor in humus and nutrients.

Preluvosols, have a spread of 18%, have a humus content of 1.7-1.8%, the nitrogen content is 0.119, that in mobile phosphorus between 10.5-11.4 mg/100 g and that in assimilable potassium is between 9.6 and 12.0 mg/100 g soil.

Ground water is found at depths of more than 15 m.

Figure 4 shows the main chemical properties of the soils in the Tirol Viticultural Center (0-20 cm).

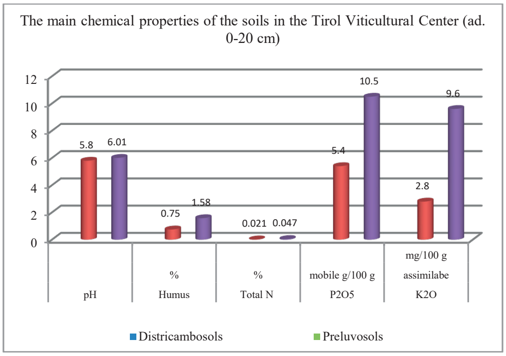

Figure 4. The main chemical properties of the soils in the Tirol Viticultural Center (0-20 cm)

The pedo-climatic conditions in this viticultural center are favorable for the cultivation of vines, the ones that cause problems are the late spring frosts, which occur less often and during the vegetation period, the ones that have a high frequency are the late frosts (both those in spring and and those in autumn), as well as the hail that in recent years has had an increasingly high frequency, which has led to a decrease in production and a decrease in the quality of the grapes. The most cultivated varieties are Fetească, Muscat, Merlot, Riesling and Sauvignon blanc.

CONCLUSIONS

The efficient use of the geographical and climate resources in this area, interwoven with the adoption of higher cultural and processing technologies to meet the demands of both domestic and foreign consumption are the priorities of modern viticulture in the Banat area.

Knowing the relationships between the plant and its development environment creates the possibility of expanding and optimizing the grapevine culture in various areas with appropriate natural conditions. The relationship between grapevines and climate factors determines the distribution of this culture in areas where it is possible.

The relief is of vital importance in determining the quality of the wine. The hilly relief increases the area of the land, and it is a barrier to the winds and the cold or hot currents. The slope also ensures the leakage of excess water, favouring the heating of the soil.

The four vineyards and viticultural centers belonging to Banat's vineyards represent an important and recognized grapevine-growing area due to its exceptional wines acknowledged both nationally and internationally (Recaș wines). Recaș wine center occupies the largest area cultivated with vines, 1589 ha.

The vineyards of Banat are represented by a series of soils, the most widespread are the luvosols that were identified in all 4 vineyards and viticultural centers and which are soils that lend themselves to this culture, followed by eutricambosols that were identified in the Moldova Nouă Viticultural Center and Recaș, regosols (Moldova Nouă), preluvosols in Recaș and Silagiu, districambosols in Tirol and antrosols in Silagiu. The soils from the Recaș Vineyard are the ones that lend themselves best to this culture, followed by those from the Silagiu Viticultural Center.

We can thus state that this part of Romania, duet to its soil and climate conditions, provides favourable and very favourable natural conditions for the culture of grapevines, especially of the varieties for high quality wines. The geographical location and infrastructure superior to the other grapevine areas of Romania have attracted many investors especially in recent years, which have contributed to the relaunch of the viticulture in this area. As a result of the investments made after 2000, numerous wine holdings appeared, which replaced the aging plantations with young plantations.

In most of the studied viticultural centers, however, there are a number of disadvantages regarding the efficiency of farms because of the exaggerated fragmentation of the property, especially in Silagiu-Buziaş, which prevents the formation of larger farms with an appropriate equipment in order to be able to practice high-performance technologies

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RESEARCHERS ON THE INFLUENCE OF THE SOWING TIME ON THE BIOLOGY AND CULTIVATION TECHNOLOGY OF THE *Dracocephalum moldavica* species, UNDER THE CONDITIONS OF INCDSCZ BRASOV

Sorina NIȚU (NĂSTASE)[1], Răzvan-Damian NIȚU[2], Emilia CONSTANTINESCU[3]

[1]National Institute of Research and Development for Potato and Sugar Beet Braşov, 2 Fundaturii Street, 500470, Brasov, Romania
[2]University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 3-5 Calea Manastur, 400372, Cluj-Napoca, Romania
[3]University of Craiova, Faculty of Agronomy, Libertatii Street, Craiova, Dolj County, Romania

Corresponding author email: emiliaconst2000@yahoo.com

Abstract

The main aim of this research was to improve the knowledge of cultivation of Dracocephalum moldavica L., by studying some aspects of biology and technology, adaptability to environmental conditions; the studies were carried out at NIRDPSB Brasov, within the Laboratory of Technology and Good Agricultural Practices, Department of Medicinal and Aromatic Plants. Originally cultivated in Central Asia and acclimatized in Central and Eastern Europe, Dracocephalum moldavica L. is traditionally used for medicinal and aromatic purposes and is also a valuable honey plant. In order to determine the optimal sowing time for Dracocephalum moldavica L., an experiment with 5 planting variants was set up in three replications, following the randomized block method, during 2015 - 2018. The area of a plot was 7.5 m^2, the experimental area including paths was 142.5 m^2; the number of plants per variant was 60 plants, and the total per experiment was 900 plants.

Key words: *Dracocephalum moldavica L., biology, conditions, planting, tehnology.*

INTRODUCTION

Romania, due to its geographical position and pedoclimatic conditions, presents a diversified flora, containing over 3600 species of superior plants, spontaneous and cultivated, of which 10-12% are used by traditional and scientific medicine.

Due to the high content of active principles, plant materials are requested by the pharmaceutical, cosmetic, dermatological industries and external manufacturing companies. The need for the cultivation of medicinal and aromatic plants is based on the hypothesis that the spontaneous flora cannot provide the necessary plant raw material for the phytotherapeutic industry, which is in full ascension.

The current guidelines in medicine are aimed at the large-scale use of phytotherapy, limiting the use of synthetic drugs to what is strictly necessary.

In this context, a paradoxical phenomenon is manifested: while the use of medicinal plants is in a vertiginous ascent (both as raw material in the drug industry and in simple forms, as natural remedies), fewer and fewer are those who use them I can also recognize the harvest. (Crăciun et al., 1988).

The introduction into culture also aims to solve some inconveniences such as: different species grow wildly, on large areas, often difficult to access, so that recognition, collection and transport are difficult. These problems postpone the optimal harvest time and lead to an increase in the cost price of the raw material. The impurity of the raw material and the harvesting of a product poor in active principles is determined by the lack of knowledge of the optimal harvesting moment, the use of casual or non-specialized personnel in this field. (Muntean, 1990; Păun et al., 1996). Romania, in the last five years, has experienced significant changes in the areas cultivated with medicinal plants: in 2016, the largest area cultivated with medicinal plants was approximately 4395 ha with a total production of 5627 t; these surfaces decreased by 27% in 2017 and drastically reduced by 61% between 2018 and 2020 (https://www.madr.ro).

Development of a cultivation technology for species *Dracocephalum moldavica* was determined by the need to introduce numerous species of medicinal plants into the therapeutic arsenal of homeopathic medicine (Alexandriu-Peiulescu, 1978; Muntean, 1990).

The research undertaken in the field of medicinal plant cultivation solves some issues related to: protecting natural pools with medicinal species from spontaneous flora, ensuring the maintenance of the natural gene pool and avoiding irrational harvesting; the maintenance of spontaneous flora of plants considered monuments of nature; the need to cultivate species whose vegetable raw material presents high toxicity, these being systematically eliminated from meadows; the cultivation of some species that are not found in the spontaneous flora, but which are requested by the pharmaceutical industry; carrying out the harvesting work, when the maximum content in active principles is registered; drying the product immediately after harvesting or processing the raw vegetable material in a fresh state, by means of special installations; providing the medicine industry with raw material, homogeneous in terms of chemical composition, eliminating the shortcomings due to the phytochemical variability of raw material harvested from spontaneous flora; the acclimatization of new species, which do not grow spontaneously in our country (Muntean, 1998).

Cultivation of improved populations and varieties, their placement in appropriate pedoclimatic conditions and the application of high-performance culture technologies, lead to higher yields, compared to spontaneous flora.

By applying ecological technologies of differentiated culture, taking into account the biology and the relationship of plants with vegetation factors, large productions of raw material, with a high content of active principles, were obtained.

Thus, ecological agriculture is based on a series of objectives and principles, as well as common practices aimed at minimizing human impact on the environment, while ensuring that the agricultural system works as naturally as possible (Savescu et al., 2016).

Originally cultivated in Central Asia and acclimatized in Central and Eastern Europe, the species *Dracocephalum moldavica* L. is traditionally used for medicinal and aromatic purposes, being also a valuable honey plant.

Dracocephalum. moldavica L. (in popular language, mătăciune) is an herbaceous, annual plant, with a straight stem, 30-70 cm tall, four-sided, branched from the base, covered with absorbent bristles, often reddish; the leaves are opposite, oblong-lanceolate, divided into 3-7 narrow segments, short petioled, with crenellated edges and glandular points on the lower face; the flowers are blue-violet or white, grouped in 5-10 clusters; the fruits are ovoid, brown tetranucles. (https://www.remediilenaturii.ro).

Dracocephalum moldavica L. has been used for centuries in alternative medicine due to its sedative and analgesic, antirheumatic, antitumor and antioxidant properties; it is used in the form of infusions or teas to calm colic, nervous states, producing a peaceful sleep. The raw material used is represented by the aerial parts (grass), harvested during the flowering period.

The aerial part is rich in active principles: flavonosides, tannins, bitter substances, mineral salts, essential oil. The volatile oil content varies between 0.08-0.78% in fresh herb and between 0.23-2.80% in dry herb (Мустяце, 1988; Verzea, 1986; Котуков, 1964).

Principal compounds of volatile oil *Dracocephalum moldavica* are very similar to those of evening primrose oil (*Melissa oicinalis* L.); it is rich in citral (30-50%), geraniol (10-14%), nerol, limonene (Verzea, 1986). The composition of the volatile oil also includes: citronellol, thymol, linalool, linalyl acetate, geranyl acetate, niril acetate and some sesquiterpenes, lavonoid (moldavoside), caffeic and succinic acid (Bojor, 2003).

As a result of the pharmacodynamic actions (astringent, tonic-aperitif, antispasmodic, antiseptic, choleretic-collagogic, relaxing, carminative, anticolytic, antiemetic) determined by the active principles, the aerial part of the wormwood is used in the treatment of colic of various etiologies, insomnia, spasms abdominal pain, nausea and vomiting, eye diseases, neuroses). It is used in the cosmetic industry to manufacture perfumes, soaps and detergents, in the food industry to flavor soft drinks, syrups, compotes, jams, canned fish, and in the pharmaceutical industry to obtain vitamin A. Is a honeydew species, with a high economic contribution - up to 200 kg/ha of honey. It can also be cultivated in dendrological parks.

The species *Dracocephalum moldavica* L. reacts well to the administration of nutrients, both to basic fertilization and in the case of the application of foliar fertilizers, an aspect highlighted by the increase in production, but also from a qualitative point of view, referring to the active principles found in the leaves of this species. The application of fertilizers - an important sequence of the cultivation technology influences the photosynthesis process, which generates the growth of the plant's organs, in different phenophases of vegetation. The percentage variation of oil production and of some compounds from the essential oil of *Dracocephalum moldavica* L., can be attributed to the composition of fertilizers, but also to pedoclimatic conditions, agricultural techniques or ecological factors; taking into account all these factors it can be said that evening primrose oil can change its composition, the main compounds identified being geranyl acetate, geranial, neral, followed by geraniol and neryl acetate. The difference in composition is revealed by other studies, which highlighted other main compounds such as citral, linaalol (Onofrei, 2017).

MATERIALS AND METHODS

The studies were carried out at I.N.C.D.C.S.Z. Brașov, within the Laboratory of Technology and Good Agricultural Practices, Department of Medicinal and Aromatic Plants.

The experiments were located in the ecological system (45°42′ N and 25°45′ E, altitude 520 m); the soil texture in the upper horizons is sandy and loamy-sandy, and at depth it is sandy-loamy with a pH of 6.7. The soil of the experimental site contains 27% clay, 4.68% humus, total nitrogen 3.15%, P_2O_5 32.1 ppm (7.36 mg 100 g soil $^{-1}$) and K_2O 105.1 ppm (12.67 mg 100 g soil $^{-1}$) (Moldovan et al. 2021).

Dracocephalum moldavica L. prefers creeping as precursor plants. Being a species sensitive to weeding, it is sown after plants that leave the land clean of weeds. It is recommended to avoid placing crops after plants from the same botanical family (*Lamiaceae*).

Land preparation. A deep autumn plowing was carried out (25-30 cm); the furrows were left under the action of freezing and thawing, and in the spring, a few days before sowing, the seed bed was prepared with a disc harrow, followed by a harrow with adjustable tines.

Maintenance works. After the emergence of the plants, the weeds were weeded in rows and between the rows, and until the flowering stems appeared, 3 weedings were carried out with the motor cultivator.

In order to establish the optimal sowing season for the *Dracocephalum moldavica* L. species, between 2015 and 2018, monofactorial experiments were set up, with 5 sowing seasons (variants), in three repetitions, according to the randomized block method (Table 1).

The surface of a plot was 7.5 m^2, the experimental surface, including the paths, was 142.5 m^2; the number of plants per variant was 60 plants, and the total per experience 900 plants. The seed material (seed) was purchased from SCDA Secuieni.

Table 1. Sowing date to experience on determining the optimal time to sow to species *Dracocephalum moldavica* L. (Brașov 2015-2018)

Sowing variant	2015-2016	2016-2017	2017-2018
V1 - sown in October	20.10.2015	24.10.2016	21.10.2017
V2 - sown at the beginning of November	11.11.2015	07.11.2016	08.11.2017
V3 - sown in emergency I (beginning of April)	06.04.2016	10. 04.2017	04.04.2018
V4 - sown in emergency II (second decade of April)	18.04.2016	17.04.2017	19.04.2018
V5 - sown in the first decade of May	05.05.2016	08.05.2017	03.05.2018

RESULTS AND DISCUSSIONS

The studies and research carried out between 2015 and 2018 at the Brașov National Research and Development Institute for Potatoes and Sugar Beet, the Laboratory of Technology and Good Agricultural Practices, the Department of Medicinal and Aromatic Plants, regarding the optimal sowing period of the species *Dracocephalum moldavica* L., have shown that this species finds good climate and soil conditions for growth and development.

The phenological observations of the experience regarding the optimal sowing period reveal the fact that the physiological processes that take place in the plant are directly influenced by the climate and environmental conditions, as well as the sowing period.

Table 2 and Figure 1, shows the results of the germination of the plants depending on the time of sowing: in the variants V1, V2 (V1 - sown in October, V2 - sown at the beginning of November), the plants did not germinate or those that germinated did not survive the winter, V3 - sown in emergency I (beginning of April) emerged, in 2016, 14 days after sowing, in 2017 at 15 days, and in 2018 at 14 days. In the case of the variants sown in emergency II (V4), the plants sprouted in 2016 after 11 days, in 2017 after 10 days, and in 2018 after 11 days after sowing. V5 - sown in the first decade of May, it sprouted in 11 days in 2016, in 2017 in 14 days, and in 2018 in 12 days.

Table 2. The date of emergence of the plants/variations to the experience regarding the establishment of the optimal time to sow at species *Dracocephalum moldavica* L. (Brașov 2015-2018)

Plant emergence date/variant	2015-2016	2016-2017	2017-2018
V1 - sown in October	-	-	-
V2 - own at the beginning of November	-	-	-
V3 - sown in emergency I (beginning of April)	20.04.2016	25.04.2017	20.04.2018
V4 - sown in emergency II (second decade of April)	29.04.2016	27.04.2017	30.04.2018
V5 - sown in the first decade of May	16.05.2016	22.05.2017	15.05.2018

From the date of sowing to the emission of flower stalks, the average of the three years was 88 days for V3, 85 days for V4 and 78 days for V5.

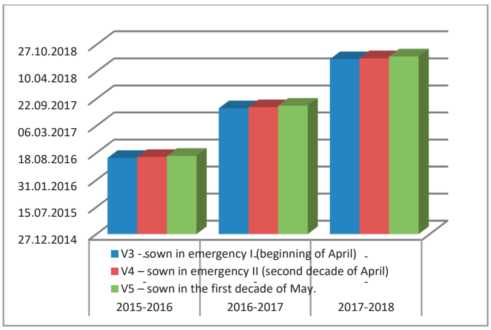

Figure 1. The date of emergence of the plants/variations to the experience regarding the establishment of the optimal time to sow at species *Dracocephalum moldavica* L. (Brașov 2015-2018)

The obtained results point out that the date of sowing does not greatly influence the physiological processes of plant development, the emission of flowering stems occurring approximately in the same period, being directly determined by climatic factors (Table 3 and Figure 2).

Table 3. The date of issuing the flower stalks/variant to the experience regarding the establishment of the optimal time to sow at species *Dracocephalum moldavica* L. (Brașov 2015-2018)

Date of issue of flower stalks/variant	2015-2016	2016-2017	2017-2018
V1 - sown in October	-	-	-
V2 - sown at the beginning of November	-	-	-
V3 - sown in emergency I (beg. of April)	04.07.2016	06.07.2017	02.07.2018
V4 - sown in emerg. II (second decade of April)	11.07.2016	14.07.2017	10.07.2018
V5 - sown in the first decade of May	20.07.2016	25.07.2017	23.07.2018

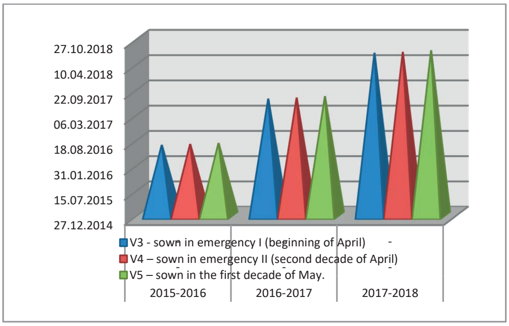

Figure 2. The date of issuing the flower stalks/variant to the experience regarding the establishment of the optimal time to sow at species *Dracocephalum moldavica* L. (Brașov 2015-2018)

From the date of emergence to full flowering, V3 - sown in emergency I (beginning of April) recorded, in 2016, 83 days, in the second year 80 days, and in the third year 83 days. In the case of the variants sown in emergency II (V4), the plants bloomed in 2016 at 81 days, in 2017 at 86 days, and in 2018 at 80 days after emergence. V5 - sown in the first decade of May bloomed in 72 days in 2016 and 2017, and in 2018 in 78 days (Table 4 and Figure 3).

Table 4. Flowering date/variant to experience regarding the establishment of the optimal time to sow at species *Dracocephalum moldavica* L. (Brașov 2015-2018)

Flowering date/variant	2015-2016	2016-2017	2017-2018
V1 - sown in October	-	-	-
V2 - sown at the beginning of November	-	-	-
V3 - sown in emergency I (beginning of April)	12.07.2016	14.07.2017	12.07.2018
V4 - sown in emergency II (second decade of April)	19.07.2016	22.07.2017	19.07.2018
V5 - sown in the first decade of May	27.07.2016	02.08.2017	01.08.2018

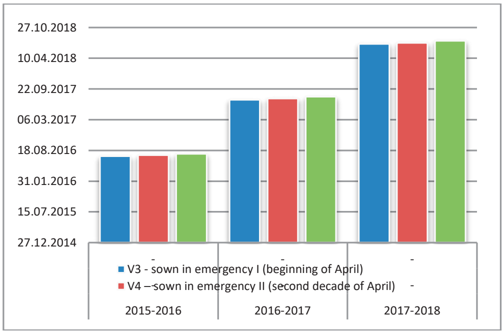

Figure 3. Flowering date/variant to experience regarding the establishment of the optimal time to sow at species *Dracocephalum moldavica* L. (Brașov 2015-2018)

For the duration of the vegetation period in the species *Dracocephalum moldavica* L., from emergence to the beginning of fruiting, the average of the three years was 89 days for V3 and V4, and for V5, 80 days (Table 5 and Figure 4).

Table 5. The date of the beginning of fruiting/the variant to the experience regarding the establishment of the optimal time to sow at species *Dracocephalum moldavica* L. (Brașov 2015-2018)

The date of the beginning of fruiting/the variant	2015-2016	2016-2017	2017-2018
V1 - sown in October	-	-	-
V2 - sown at the beginning of November	-	-	-
V3 - sown in emergency I (beginning of April)	19.07.2016	20.07.2017	20.07.2018
V4 - sown in emergency II (second decade of April)	26.07.2016	28.07.2017	27.07.2018
V5 - sown in the first decade of May	02.08.2016	09.08.2017	07.08.2018

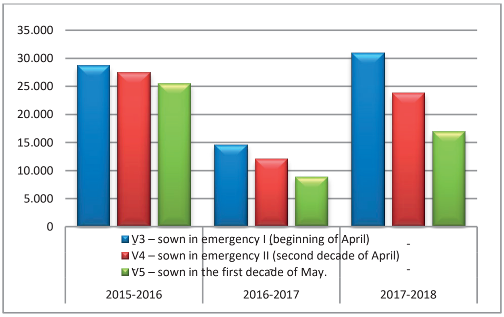

Figure 4. The date of the beginning of fruiting / the variant to the experience regarding the establishment of the optimal time to sow at species *Dracocephalum moldavica* L. (Brașov 2015-2018)

Table 6 shows the average productions of fresh grass per ha/variety, during the experimental years. V3 - sown in emergency I (beginning of April) recorded an average production of 24,763 kg/ha, V4 - sown in emergency II (second decade of April) 21,129 kg/ha, and V5 - sown in the first decade of May 17,111 kg/ha (Figure 5).

Table 6. The production of fresh grass (kg/ha/variant) to the experience regarding the establishment of the optimal season for sowing at species *Dracocephalum moldavica* (Brașov 2015-2018)

Production of fresh grass (kg/ha/variety)	2015-2016	2016-2017	2017-2018	Average (kg/ha)
V1 - sown in October	-	-	-	-
V2 - sown at the beginning of November	-	-	-	-
V3 - sown in emergency I (beginning of April)	28,716	14,613	30,960	24,763
V4 - sown in emergency II (second decade of April)	27,493	12,053	23,840	21,129
V5 - sown in the first decade of May	25,493	8,880	16,960	17,111

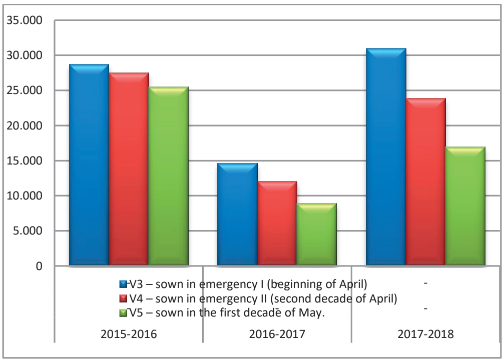

Figure 5. The production of fresh grass (kg/ha/variant) to the experience regarding the establishment of the optimal season for sowing at species *Dracocephalum moldavica* L. (Brașov 2015 - 2018)

CONCLUSIONS

The general objective of this study was the introduction into culture of some species of medicinal and aromatic plants of phytotherapeutic interest and the maintenance of biodiversity in these species. This objective resulted from the increased concerns of growers, processors, consumers of medicinal and aromatic plants, aiming to return to nature in terms of human nutrition and health preservation with products of an ecological nature and the increased responsibility for protecting the environment and natural habitats of these plants.
The research on the influence of the sowing time on the biology and cultivation technology of the species *Dracocephalum moldavica* L., in the conditions of the National Research and Development Institute for Potato and Sugar Beet Brașov, the Laboratory of Technology and Good Agricultural Practices, the Department of Medicinal Plants and Aromatic, they showed that this species finds good climate and soil conditions for growth and development.
Studies have shown that the species *Dracocephalum moldavica* L. needs a higher temperature for germination, being sensitive to late frosts; it is recommended to sow in spring, in emergency I. Some authors recommend as sowing times, both the one in spring and the one on the threshold of winter (https://www.agrimedia.ro), sowing in the "threshold of winter" being advantageous from an organizational point of view as well, having place in the period when less agricultural work is carried out, compared to the spring season. This era also presents disadvantages due to the alternation of favorable periods for germination and emergence, with frosty periods, when the young seedlings are destroyed (Constantinescu, 2009).
In the depressed area of Brasov, where the experiments took place, autumn sowing is not recommended; the seeds do not have favorable temperatures for germination and, where they manage to sprout, they perish in the winter due to the very low temperatures. In the depressed area of Brasov, where the experiments were located, autumn sowing is not recommended; the seeds do not have favorable temperatures for germination and, where they manage to sprout, they perish in the winter due to the very low temperatures.
The best herb production of the species *Dracocephalum moldavica* L. at I.N.C.D.C.S.Z. Brașov was obtained during spring sowing, in emergency I, at the beginning of April, when the highest values of the plant mass were determined, with an average production of the 3 experimental years of 24,763 kg/ha .The contribution with new information on the crop technology of *Dracocephalum moldavica* L. species leads at the enrichment of knowledge in this field, as the information in the literature is more geared towards studying the species in terms of active principles in the plant, rather than crop technology (Nitu et al., 2017). The results obtained can also be used by agronomists, pharmacists and biologists, who, directly or indirectly, contribute to the cultivation and use of medicinal and aromatic plants.

ACKNOWLEDGEMENTS

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THE INFLUENCE OF LONG-TERM ANTHROPOGENIC LOAD ON THE MIGRATION OF MOBILE ALUMINUM COMPOUNDS, PHYSICAL AND CHEMICAL PROPERTIES OF ALBIC STAGNIC LUVISOL

Yuriy OLIFIR, Anna HABRYEL, Oleh HAVRYSHKO, Tetiana PARTYKA, Hryhoriy KONYK, Nadiia KOZAK

Institute of Agriculture of Carpathian Region of National Academy of Agrarian Sciences of Ukraine, 5 Hrushevskoho Street, Obroshyne, 81115, Lviv Region, Ukraine

Corresponding author email: olifir.yura@gmail.com

Abstract

FAO singles out acidic soils, which are widespread in Ukraine, as problematic soils. At the same time, the role of aluminium in soil acidity formation and aluminium toxicity is becoming increasingly important. The study was conducted in a long-term stationary experiment and under conditions of natural analogues of Albic Stagnic Luvisol under forest and fallow land. It was found that cultivation and long-term use of different doses of fertilizers and lime change the content of mobile aluminium compounds, physical and chemical properties of not only the upper humus horizons but also affect the lower horizons and soil formation processes. The highest content of mobile aluminium compounds, 110.3-121.5 mg/kg of soil, is accumulated in the upper humus horizons in the control without fertilizers. With prolonged mineral fertilization, the highest content of mobile aluminium compounds (148.1 mg/kg) is concentrated in the illuvial-eluvial horizon. In the soil under the forest, the highest content of mobile aluminium is accumulated in the upper humus horizons AEg, Ehg and is 210.6 and 183.0 mg/kg of soil, respectively, with the lowest pH_{KCl} value of 3.72 and the highest hydrolytic acidity of 9.73 mg-eq/100 g of soil. The content of mobile aluminium ranges on the fallow land from 54.3 in AEg to 55.7 mg/kg in the Cg horizon, i.e. it is characterized by its uniform distribution.

***Key words**: acidity, aluminium, Albic Stagnic Luvisol, fertilizers, liming.*

INTRODUCTION

Among the sectors of the economy, agriculture reacts most noticeably to the climatic changes of recent decades. The future of Ukraine's food security will depend on how it adapts to the transformation of weather conditions. Among the problematic soils in terms of their agricultural use, the FAO particularly highlights acidic soils, which are widespread in Ukraine (FAO & ITPS, 2015; Tkachenko & Boris, 2021). The transition to new forms of farming, climate change, increased acidity, and the use of a disproportionate ratio of fertilizers in conditions of intensive agricultural production is leading to increased soil and land cover degradation (Lykhochvor et al., 2022).

Soil acidification is becoming a growing problem for food security due to the formation of free aluminium ion (Al^{3+}), which is toxic to crops (Li et al., 2022).

However, the main goal of chemical amelioration is not to completely neutralize acidity, but primarily to reduce the level of exchangeable Al^{3+} and increase the level of exchangeable Ca^{2+} in the soil (Almaliev, 2022). The dominant aluminium (Al^{3+}) in soil composition has a significant impact on the establishment of acid-base balance in soils, thereby playing an important role in shaping fertility. Its content varies significantly in soils of different compositions and genesis, depending on the parent material. In podzolic and sod-podzolic soils, aluminium is 1.5-2.0 times higher in the illuvial (B) than in the eluvial (E) horizons (Kyrylchuk & Bonishko, 2011).

The importance of Al in soil formation and soil fertility is very high. Firstly, aluminium plays a constitutional role, as aluminosilicates are the most abundant soil minerals - they make up nearly 85% of the Earth's crust. Secondly, Al is quite reactive and forms various compounds that migrate along the profile (Nazarenko et al., 1998). At the same time, aluminium participates in the formation of potential soil acidity (exchangeable and hydrolytic), which adversely affects the growth and development of crops. Meanwhile, the Al content affects plant nutrition by forming hardly soluble aluminium phosphates, whose phosphorus is not available to plants (Tkachenko et al., 2019). According to studies (Godsey et al., 2007; Brown et al., 2008), the physical and chemical properties of soils are significantly impaired by the presence of aluminium cations.

The total concentration, composition and availability of aluminium in the soil depend on the pH and chemical environment of the solution (Bojorquez-Quintal et al., 2017; Smirnov & Taran, 2013). Al compounds are immobile in slightly alkaline and neutral environments, but gain mobility in an acidic environment, forming soluble organo-mineral complexes with fulvic acids, which causes its active migration along the profile. The resulting organic-aluminium complexes undergo various transformations depending on environmental conditions. In the soils of the podzolic zone, they are fixed in the composition of humic substances. With increasing pH, these complexes usually decompose and Al is precipitated as hydroxides (Smirnov & Taran, 2013). In studies (Schroder et al., 2011), soil acidification below pH 5.0-5.5 increases the solubility of Al^{3+} in the soil and negatively affects the growth and yield of crops. In addition, the level and toxicity of Al increase dramatically (Gillespie et al., 2021).

In studies (Szara et al., 2019), liming in combination with mineral fertilizers reduced the sorption capacity in the entire sandy soil profile, especially as a result of the fixation of amorphous Al and Fe (hydro)oxides into more crystalline forms.

In acidic soils with a pH<5.0, phytotoxic aluminium (Al^{3+}) quickly inhibits root growth and subsequently negatively affects water and nutrient uptake by plants. This is one of the reasons why with an increased aluminium concentration in the soil profile the roots are predominantly located in the upper layer with a lower aluminium content, which is an important part of the crop's resistance to acidic soils (Smirnov et al., 2020).

According to research (Souza et al., 2023), exchangeable aluminium is significantly affected by nitrogen fertilizers, which lower the soil pH. At the same time, a higher content of organic matter on the soil surface reduces the concentration of mobile Al even at a lower pH.

The main research objective is to determine the migration of mobile aluminium compounds on Albic Stagnic Luvisol under different anthropogenic loads compared to primary soil formation under forest and fallow land (as an analogue of natural virgin soils) and to study their impact on soil formation processes.

MATERIALS AND METHODS

Long-term stationary experiments provide in-depth information on the origin of soil acidity and the role of aluminium in its formation, as well as aluminium toxicity

Research work was carried out in the classic stationary experiment of the Laboratory of Agrochemistry of the Institute of Agriculture of Carpathian region of the National Academy of Agrarian Sciences (49°47'54.3"N 23°52'26.9"E), established in 1965.

The stationary experiment consists of three fields. Each of the fields has 18 treatments in three repetitions. The arrangement of variants is single-tiered and sequential. Plot's total area is 168 m^2, and the accounting area is 100 m^2. The experiment uses a four-field crop rotation with the following crops: corn for silage - spring barley with underplanting of red clover - red clover - winter wheat. For soil cultivation, crop cultivation and crop care, we used technologies customary for the conditions of the Western Forest-Steppe zone.

The soil of the experimental site is Albic Stagnic Luvisol (WRB, 2015) (light-grey forest surface-gleyed soil by Ukrainian classification). Its arable (0-20 cm) layer had the following averaged initial fertility indicators: humus content (by Tyurin) 1.42%, pH_{KCl} 4.2, hydrolytic (by Kappen) and exchangeable acidity (by Sokolov) - 4.5 and 0.6 mg-eq/100 g of soil and 60.0 mg/kg of mobile aluminium. The content of mobile phosphorus (by Kirsanov) and exchangeable potassium (by Maslova) was 36.0 and 50.0 mg/kg of soil respectively.

As organic fertilizer semi-rotted cattle manure on straw bedding was used. Mineral fertilizers consisted of granular superphosphate (19.5%), potassium salt (40%), ammonium nitrate (34.5%) and nitroammophoska (NPK 16% each). NPK content was balanced with simple fertilizers when using nitroamophoska. Manure as organic fertilizer was applied under corn (40-60 t/ha). In the fall, phosphorus and potash fertilizers were applied during the main ploughing. Nitrogen fertilizers were used during pre-sowing cultivation in the spring. Before the start of the 9th rotation of crop rotation, liming was carried out, during which the doses of lime were adjusted. Limestone flour (93.5% $CaCO_3$) was used as limestone materials. The second mowing of red clover was ploughed as an organic fertilizer in all treatments starting from the 8th rotation.

The study of changes in the content of mobile aluminium was carried out in the soil profiles under the forest, on the fallow and the most representative treatments of the stationary experiment: absolute control (without fertilization), organo-mineral fertilization system (10 t/ha of manure per crop rotation area + $N_{65}P_{68}K_{68}$) with periodic liming with 6.0 t/ha of limestone flour (1.0 n $CaCO_3$ by hydrolytic acidity) and mineral fertilizer system ($N_{65}P_{68}K_{68}$).

Soil samples for determination of physical and chemical properties were collected on the studied variants by genetic horizons of Albic Stagnic Luvisol and prepared for analysis following DSTU ISO 11464-2001. Determination of pH_{KCl} was carried out by the potentiometric method at a ratio of soil to 1.0 n KCl solution 1:2.5 using a pH meter "pH-301" (DSTU ISO 10390:2007). Hydrolytic acidity (Ha) was determined by the titrimetric method in the extract of a 1.0 n CH_3COONa solution at a soil:solution ratio of 1:2.5, shaking for 1 hour and titration with a 0.1 n NaOH solution.

The content of mobile aluminium was determined by the Sokolov method (extraction with 1.0 n KCl (1:2.5), shaking for 1 h, followed by titration after boiling for 5 min in a hot state with a 0.02 n NaOH solution.

The research data was processed using OriginPro 2019b software (OriginLab Corporation, USA, 2019). A comparison of the obtained data was conducted using the Tukey test ($p<0.05$). Data are presented as an arithmetic mean with standard deviation ($x \pm SD$).

RESULTS AND DISCUSSIONS

Studies conducted in a stationary experiment have shown that long-term ploughing and the use of different doses of fertilizers and lime on an Albic Stagnic Luvisol (compared with the primary soil formation under the forest and fallow - analogues of natural virgin soils) changes the content of mobile aluminium compounds, physical and chemical properties of the upper humus horizons. At the same time, it has an intense impact on the horizons below, thereby affecting soil formation processes that occur under different anthropogenic loads.

The obtained indicators of the physicochemical characteristics of the control and fallow land variants indicate the low agroecological quality of Albic Stagnic Luvisol due to high acidity.

Characterizing the distribution of mobile aluminium in the soil profile, it should be noted that with the introduction of different doses of fertilizers and lime, the largest amount of it is concentrated in the illuvial weakly eluviated gleyed horizon (Beg). At the same time, the highest value of 148.1 mg/kg of soil mobile aluminium in the profile was determined by the long-term application of only mineral fertilizers (Table 1).

With long-term use of the organo-mineral fertilizer system and liming, the content of mobile aluminium in the humus-eluvial arable horizon is the lowest - 26.1 mg/kg. This is because, with a slightly acidic reaction of the soil solution (pH_{KCl} - 5.18), the content of organic matter in the upper horizon increased to 1.90%, which contributed to a decrease in the concentration of mobile aluminium and a reduction in its toxicity due to the formation of aluminium-humus complexes. Similar results were obtained in studies (Li et al., 2022), where the presence of more organic matter in the soil not only improved their acid buffering capacity but also suppressed the accumulation of exchangeable and soluble Al.

With the depth of the soil profile under this fertilization system, the content of mobile aluminium in the humus-eluvial subsoil, eluvial, and weakly eluvial horizons increases to 64.8-79.7 mg/kg. In the lower horizons, the content of mobile aluminium compounds decreases more than twice to 24.3-43.2 mg/kg in parallel to the increase in the number of absorbed bases to 18.9-16.3 mg-eq/100 g of soil.

This is primarily due to a small amount of organic matter, represented mainly by mobile fulvic acids, with which aluminium forms complex organo-mineral compounds.

Table 1. Changes in the physical and chemical properties of the genetic horizons of the Albic Stagnic Luvisol under the long-term anthropogenic influence, the end of the 9th rotation (x ± SD, n = 6)

Genetic horizons	Sampling depth, Cm	pH_{KCl}	Hydrolytic acidity	A sum of absorbed bases	Mobile aluminium, mg/kg of soil	Humus, %
			mg-eq/100 g of soil			
1	2	3	4	5	6	7
No fertilizers (control)						
AEg (arable)	0-18	4.22±0.14a	5.11±0.59a	3.0±0.1a	110.3±15.3a	1.48±0.04a
AEg (sub-arable)	18-31	4.18±0.37a	4.94±0.10a	2.4±0.6a	121.5±9.8a	1.40±0.07a
Ehg	31-64	4.31±0.09a	3.58±0.42a	5.2±0.5a	65.3±1.9a	0.48±0.03a
Beg	64-110	4.13±0.18a	4.20±0.17a	10.4±1.2a	91.8±0.2a	0.28±0.02a
Bg	110-131	4.22±0.08a	3.23±0.10a	6.9±0.1a	68.4±1.7a	0.28±0.01a
BCg	131-180	4.47±0.03a	1.40±0.04b	6.0±0.7a	27.5±2.0a	0.47±0.02a
CBg	180-200	4.35±0.07a	2.62±0.44a	8.0±0.1a	36.5±2.6a	0.26±0.02a
	LSD_{05}	*0.15*	*1.72*	*3.66*	*46.50*	*0.70*
Organo-mineral fertilizer system ($N_{65}P_{68}K_{68}$ + 10 t/ha of manure + $CaCO_3$ 1.0 n by Ha)						
AEg (arable)	0-20	5.18±0.10b	2.77±0.22b	10.6±0.5b	26.1±1.4b	1.90±0.05b
AEg (sub-arable)	20-35	5.05±0.07b	2.86±0.15b	7.5±0.5b	64.8±0.1b	1.61±0.16b
Ehg	35-55	4.90±0.12b	3.11±0.05a	8.5±0.2b	71.1±9.3a	0.83±0.15b
Beg	55-81	4.78±0.21b	3.46±0.21a	9.0±1.9a	79.7±1.6a	0.64±0.10b
Bg	81-150	4.90±0.13b	3.15±0.04a	11.9±0.8b	43.2±5.8b	0.55±0.06b
BCg	150-193	4.85±0.06a	2.98±0.01a	16.3±1.2b	32.4±4.2a	0.51±0.00a
CBG	193-215	4.87±0.12b	2.95±0.04a	18.9±0.1b	24.3±2.1a	0.40±0.01a
	LSD_{05}	*0.18*	*0.30*	*5.59*	*29.88*	*0.77*
Mineral fertilizer system ($N_{65}P_{68}K_{68}$)						
AEg (arable)	0-22	4.03±0.07a	5.11±0.92a	3.0±0.5a	75.2±5.1c	1.57±0.03a
AEg (sub-arable)	22-35	3.98±0.09a	5.20±0.07a	2.8±0.2a	99.5±0.9c	1.45±0.03ab
Ehg	35-61	4.17±0.04a	4.54±0.06b	1.5±0.1c	126.5±3.8b	0.63±0.06a
Beg	61-87	4.00±0.02a	5.25±0.06b	5.7±0.2b	148.1±3.0b	0.37±0.03a
Bg	87-150	4.07±0.10a	2.97±0.02a	8.0±1.3a	66.2±2.8a	0.26±0.02a
BCg	150-180	4.04±0.05b	2.97±0.10a	13.8±0.3c	54.5±6.1b	0.21±0.01b
CBG	180-200	4.11±0.04a	2.80±0.05a	15.2±0.5c	36.0±0.9a	0.31±0.02a
	LSD_{05}	*0.09*	*1.51*	*7.17*	*52.59*	*0.76*

Note. Values labelled with the same letter within one soil horizon are not significantly different from each other according to the Tukey test (p<0.05).

It is known from studies (Berggren & Mulder, 1995) that the solubility of Al in acidic mineral soil horizons is controlled by the reaction of complexation with soil organic matter. After all, the pool of organically bound soil Al strongly controls the solubility of Al in acid soil suspensions.

In the soil under the forest and the control without fertilizers, the highest content of mobile aluminium is accumulated in the upper humus horizon - 210.6 mg/kg of soil under the forest and 110.3-121.5 mg/kg in the control without fertilizers. On the fallow land, the content of mobile aluminium ranged from 54.3 mg/kg of soil in AEg to 55.7 mg/kg in the Cg horizon, i.e., its uniform distribution along the profile is characteristic (Table 2).

Studies in a stationary experiment have shown that at the end of the IX rotation of four-field crop rotation, the acidity (pH_{KCl}) of the soil profile does not change significantly. In the humus-eluvial arable horizon (AEg arable.), the pH_{KCl} is 4.22. With depth, the pH value of the salt extract changes unevenly from 4.31 in the eluvial weakly humic gleyed (Ehg) horizon to 4.13 in the illuvial slightly eluviated gleyed (Beg) and to 4.35 in the CBg horizon at a depth of 181 cm. Hydrolytic acidity decreases from 5.11 in the humus-eluvial horizon to 1.40-2.62 mg-eq/100 g of soil in the BCg and CBg horizons (Table 1).

It was established that the soil under the forest is characterized by a very strongly acidic reaction of the soil environment of all genetic horizons (3.72-3.94 units of pH_{KCl}). At the same time, the upper AEg horizon is characterized by the lowest pH_{KCl} value of 3.72 and the highest hydrolytic acidity - 9.73 mg-eq/100 g of soil (Table 2).

Table 2. Changes in the physical and chemical properties of the genetic horizons of the Albic Stagnic Luvisol under the forest and fallow (x ± SD, n = 6)

Genetic horizons	Sampling depth, cm	pH_{KCl}	Hydrolytic acidity (Ha)	The sum of absorbed bases	Mobile aluminium, mg/kg of soil	Humus, %
			mg-eq/100 g of soil			
1	2	3	4	5	6	7
Forest						
AEg	5-26	3.72±0.12	9.73±0.08	1.1±0.2	210.6±1.7	2.07±0.08
Ehg	26-47	3.86±0.04	6.49±0.01	0.9±0.2	183.0±0.9	1.23±0.06
Beg	47-64	3.78±0.02	4.51±0.04	1.0±0.1	90.1±1.3	0.53±0.07
Bg	64-96	3.83±0.01	4.60±1.14	6.8±0.2	77.7±3.4	0.33±0.05
Bcg	96-122	3.83±0.05	3.79±0.04	11.2±0.4	53.7±0.1	0.29±0.07
Cbg	122-150	3.89±0.01	3.42±0.02	11.9±0.6	48.7±1.3	0.26±0.05
Bg	150-173	3.94±0.01	3.15±0.02	13.7±0.1	37.6±1.2	0.22±0.04
	LSD_{05}	*0.09*	*3.04*	*7.44*	*90.08*	*0.91*
Fallow						
AEg	5-30	4.25±0.05*	5.59±0.02*	5.6±0.1*	54.3±2.6*	1.74±0.11*
Ehg	30-40	4.35±0.03*	3.41±0.00*	5.2±0.4*	45.3±0.1*	0.46±0.05*
Beg	40-61	4.23±0.07*	3.85±0.03*	11.2±0.8*	51.6±1.5*	0.41±0.13
Bg	61-102	4.21±0.23*	3.50±0.17*	11.4±1.1*	50.9±0.7*	0.28±0.08
Bcg	102-129	4.48±0.05*	1.31±0.02*	5.6±0.3*	20.0±2.4*	0.21±0.04
Cbg	129-150	4.32±0.14*	2.80±0.20*	7.4±0.6*	36.7±1.0*	0.26±0.03
Cg	150-180	4.23±0.27	3.58±0.30*	11.4±2.5	55.7±1.4*	0.19±0.01
	LSD_{05}	*0.13*	*1.67*	*3.88*	*16.68*	*0.72*

* - differences significant between forest and fallow soil according to Tukey test ($p < 0.05$)

It should be noted that the upper horizons (AEg, Ehg and Beg) of the soil under the forest are characterized by a very low sum of absorbed bases, which ranges from 0.9 to 1.1 mg-eq/100 g of soil. Starting from the illuvial gleyed horizon (Bg), the amount of absorbed bases increases from 6.8 mg-eq/100 g of soil in this horizon to 13.7 mg-eq/100 g of soil in the parent material (Cg) due to their enrichment with silt factions.

The Albic Stagnic Luvisol of the fallow is characterized by an acid reaction of the soil environment in all horizons where the pH_{KCl} value is below 4.5 (Table 1). With depth, the reaction of the soil solution decreases. In the illuvial glaciated horizon (Bg) it is 4.21, closer to the parent material it increases to 4.32-4.48 units.

Also, in the Albic Stagnic Luvisol under the fallow, compared to the forest, an increase in the sum of absorbed bases in the upper horizons was noted. Thus, in the humus-eluvial and eluvial horizons, it amounts to 5.6 and 5.2 mg-eq/100 g of soil, respectively, which characterizes such soils with a low degree of supply of bases. Due to the increase of silty fractions with depth in the fallow, as well as under the forest, an increase in the sum of absorbed bases to 11.4 mg-eq/100 g of soil was noted (Table 2). According to (Guo et al., 2010), soil acidification in natural conditions is slow and can be caused by decomposition of organic matter and the leaching of cations by excessive precipitation.

The Albic Stagnic Luvisol with a genetically inherent acid reaction of the soil solution in the control without fertilizers is characterized by a very low degree of the sum of absorbed bases in the upper humus horizons. Thus, in the arable and subarable layers, it is 3.0 and 2.4 mg-eq/100 g of soil, respectively. It is lower than on the fallow in the AEg horizon, which is associated with the removal of Ca and Mg by crops. This once again indicates the importance and expediency of mandatory periodic liming of acidic Albic Stagnic Luvisols. With the depth of the control soil in the lower genetic horizons, the amount of absorbed bases increases but does not differ significantly from virgin soil under forest and fallow land.

The long-term use of organic and mineral fertilizers in the crop rotation at a dose of $N_{65}P_{68}K_{68}$ + manure 10 t/ha of the crop rotation area on the background of applying 1.0 norm of lime by Ha had a positive effect on the reaction of the soil solution in all genetic horizons. This creates favourable conditions for the development and growth of crops. The pH_{KCl} value of the arable and subarable layers is 5.18 and 5.05 units, respectively. The seasonally gleyed Ehg and Beg horizons under this fertilizer are characterized by lower pH_{KCl} values of 4.90 and 4.78 units. At the same time, the hydrolytic acidity within the soil profile did not exceed 3.46 mg-eq/100 g of soil (Table 1).

Systematic application of mineral and organic fertilizers in crop rotation on the background of periodic liming with $CaCO_3$ (1.0 n by Ha) increases the sum of absorbed bases within the entire soil profile. Thus, the humus-eluvial arable horizon (AEg_{arable}) is characterized by an average degree of availability of the sum of absorbed bases, which is 10.6 mg-eq/100 g of soil. It decreases to 7.5 mg-eq/100 g of soil in the sub-arable humus-eluvial horizon ($AEg_{sub-arable}$) and increases with depth, including to 18.9 mg-eq/100 g of soil in the heavily gleyed parent material. This creates certain agroecological problems due to their leaching into groundwater.

It was found that under the mineral fertilization system, the sum of absorbed bases changed minimally in the 0-35 cm soil layer. Most of them are concentrated in the lower horizons, which is associated with the removal of a significant amount of bases by flows of matter and energy in conditions of periodic overwetting and acidification by mineral fertilizers. This distribution in the profile is also typical for the soil under forests.

Long-term application of only mineral fertilizers in crop rotation causes significant acidification of the soil solution. Therefore, the value of pH_{KCl} throughout the soil profile is in the range of 3.98-4.17 units (Table 1).

A similar pattern can be observed regarding the change in hydrolytic acidity (Ha). Long-term application of only mineral fertilizers in the recommended dose of $N_{65}P_{68}K_{68}$ without liming increased with a depth the Ha value from 5.11 to 5.25 mg-eq/100 g of soil in the Beg horizon. Changes in the pH values of saline and hydrolytic acidity can be caused not only by the long-term anthropogenic impact but also by a change in the climatic and agroecological state of the Western Forest-Steppe zone, due to increased heat and moisture supply of the territory (Poliovyi et al., 2019).

In studies (Souza et al., 2023), long-term application of mineral nitrogen fertilizers significantly reduced soil pH in the top layer (0-15 cm) and deeper soil layers, especially at higher application rates. Regardless of the depth of soil sampling, the decrease in pH was significantly related to the amount of N applied. In addition, nitrogen fertilization significantly increased exchangeable Al but decreased the sum of $Ca^{2+} + Mg^{2+} + K^{+} + Na^{+}$ throughout the topsoil and deeper layers.

In studies (Ghimire et al., 2017), soil acidification also occurred primarily on the soil îsurface up to 20 cm deep, potentially affecting nutrient dynamics, growth, development and yield of wheat.

The correlation coefficients between humus content and soil physicochemical parameters varied significantly across treatments, indicating the close nature of the relationship between them. Under the organic-mineral fertilizer system, a close correlation between humus content and pH_{KCl} (r = 0.927-0.970) was found, and the dose of lime does not affect the closeness of this relationship (Table 3). This indicates a significant role of liming in general not only for regulating the acid-base regime but also for improving the conditions of humus formation in the soil profile.

Table 3. Correlation of humus with physical and chemical parameters in the profile of Albic Stagnic Luvisol

Treatment	pH_{KCl}	Hydrolytic acidity	The sum of absorbed bases	Mobile aluminium
		mg-eq/100 g of soil		mg/kg of soil
Forest	-0.684	0.987	-0.728	0.963
Fallow	-0.229	0.791	-0.420	0.368
No fertilizers (control)	-0.301	0.697	-0.865	0.747
Organo-mineral fertilization system	0.927	-0.597	-0.570	-0.004
Mineral fertilization system	-0.386	0.711	-0.670	0.151

In the soil under the forest, close correlations were found between humus content and mobile aluminium ($r = 0.963$), and hydrolytic acidity ($r = 0.987$), as well as a close negative relationship with the sum of absorbed bases ($r = -0.728$).
This indicates the formation of organo-mineral compounds in the soil under the forest, which are associated with aluminium.
On the control without fertilizers, there is also a close correlation between humus content and mobile aluminium, hydrolytic acidity and the sum of absorbed bases, but they are weaker than under the forest (correlation coefficient is 0.747, 0.697 and -0.865, respectively). On fallow and with the introduction of only $N_{65}P_{68}K_{68}$ a close relationship was found only with hydrolytic acidity (0.791 and 0.711). Based on the results of the research, a mathematical model was created based on correlation and regression analysis that reproduces the link between the physical and chemical properties of Albic Stagnic Luvisol's genetic horizons under the forest, on the fallow and under different fertilization systems in the experiment (Table 4). The resulting equation is reliable at 95% of the probability level according to Fisher's test ($F_{fact.} > F_{05}$), and the coefficient of the equations is reliable according to Student's test. The multiple correlation coefficient given in Table 4 ($R = 0.730$-0.996) validate close relationship between the indicators included in the equations. The coefficient of determination ($D = 53.3$-99.2%) indicates a significant influence of pH_{KCl} (argument - X_1) and Ha (argument - X_2) on humus content (function - Y).

Table 4. Models of the relationship between physical and chemical properties of Albic Stagnic Luvisol

Treatment	Regression equation	R	D, %
Forest	$Y = 124.4395 – 66.5722X_1 + 8.8218X_1^2 + 0.3827X_2 – 0.0054X_2^2$	0.992	98.4
Fallow	$Y = –180.5181 + 82.6632X_1 – 9.4211 X_1^2 – 0.5715X_2 + 0.1375 X_2^2$	0.995	99.0
Organo-mineral fertilization system	$Y = –271.3405 + 126.9458X_1 – 12.3350 X_1^2 – 35.0755X_2 + 5.7108 X_2^2$	0.978	95.6
Mineral fertilization system	$Y = –529.0588 + 259.2015X_1 – 31.9601 X_1^2 + 1.8293X_2 – 0.1775 X^2_2$	0.730	53.3

Note: Y - humus content, %; X_1 - pH_{KCl}; X_2 - hydrolytic acidity (Ha), mg-eq/100 g of soil.

Therefore, the physicochemical parameters of the genetic horizons of the control without fertilizers and the mineral fertilization system indicate low agroecological quality, low fertility potential and the further development of the podzolic process in the Albic Stagnic Luvisol in the process of its agricultural use. Measures to increase fertility and counteract intra-soil degradation processes in Albic Stagnic Luvisol under the long-term anthropogenic influence are an organo-mineral fertilization system with the application of optimal doses of mineral and organic fertilizers, in particular, $N_{65}P_{68}K_{68}$ + manure 10 t/ha of crop rotation area on the background of periodic liming. This fertilization system helps to reduce the content of mobile aluminium compounds, optimize physicochemical parameters and create conditions for activating humus formation.

CONCLUSIONS

The involvement of Albic Stagnic Luvisol in agricultural use, depending on the intensity of the impact, is accompanied by changes in the content of mobile aluminium compounds and the physical and chemical properties of both humus and lower horizons. The organic-mineral fertilizer system with periodic liming, positively affecting the reaction of the soil solution ($pH_{KCl} = 5.18$, Ha = 2.37 mg-eq/100 g of soil), reduces the content of mobile aluminium compounds within the genetic profile from 26.1 in upper to 79.7 mg/kg in the Beg horizon. At the same time, the amount of absorbed bases increases to 10.6-18.9 mg-eq/100 g of soil.
Long-term use of the mineral fertilizer system, accompanied by acidification of the soil profile to pH_{KCl} 3.98-4.17, an increase in Ha to 5.11-5.25 mg-eq/100 g of soil, a decrease in the sum of absorbed bases of the upper horizons to 1.5-3.0 mg-eq/100 g of soil, increases the content of mobile aluminium to 126.5-148.1 mg/kg in the Ehg and Beg horizons to the greatest extent. On the control without fertilizers, the content of mobile aluminium is the highest in the arable and sub-arable horizons and amounts to 110.3-121.5 mg/kg of soil at pH_{KCl} 4.22-4.18, Ha 5.11-

4.94 mg-eq/100 g of soil, the sum of absorbed bases 3.0-2.4 mg-eq/100 g of soil.

In the Albic Stagnic Luvisol under the forest, the highest aluminium content is accumulated in the upper humus horizon and amounts to 210.6 mg/kg of soil. On fallow land, a uniform distribution of mobile aluminium compounds is characteristic of the Albic Stagnic Luvisol: 54.3 mg/kg of soil in AEgl to 55.7 mg/kg in the parent material (Pgl).

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SMARAGD AS NEW CHELATED-GUMATIC PREPARATION FOR IMPROVING THE ENVIRONMENTAL STATE OF THE SOIL - PLANT SYSTEM

Valentina SAMOKHVALOVA[1], Victor BUBLYK[1], Yana POGROMSKA[2]

[1]National Scientific Center "Institute for Soil Science and Agrochemistry Research named after O.N. Sokolovsky", 4 Chaykovska Street, Kharkiv, 61024, Ukraine
[2]State Enterprise "Experimental Economy Donetsk" of the National Scientific Center "Institute for Soil Science and Agrochemistry Research named after O.N. Sokolovsky", 1 Myru Street, Sukha Balka vil., Toretsk District, Donetsk Region, 85299, Ukraine

Corresponding author email: v.samokhvalova.com@gmail.com

Abstract

A new method of obtaining fertilizer and soil improver and the procedure for using the chelated-gumatic drug Smaragd with a fertilizing-stimulating and remedial effect have been substantiated and elaborated. The method aims to improve the ecological condition and increase the stability of the soil-plant system due to the creation and use of a new effective composition of the drug. New composition ensures the saturation of the soil with trace elements in a form accessible to plants, increasing plant productivity, including under conditions of heavy metal pollution, arid conditions on carbonate and eroded soils for simplifying the preparation and use of the drug and simultaneously saving resources. The new chelated-gumatic preparation of the proposed complex composition contains trace elements (Fe, Mn, Zn, Cu, Co, Mo and B) in the form of chelate compounds; the gumatic component of the composition is of natural origin. The agents of the new drug Smaragd interact in a certain ratio. The method is protected by a security document (utility model patent 135145 UA 2019).

***Key words**: Smaragd chelated-gumatic preparation, soil-plant system, remediation, microbiological and biochemical activity of soil, trace elements, heavy metals pollution.*

INTRODUCTION

Improving the ecological state of the soil-plant system by optimizing the plants mineral nutrition using the effect of humic acid, chelates, nano-chelates on the absorption of nutrients, grain yield (Najafi et al., 2020; Hayati et al., 2022) and the remediation of the polluted soil-plant system under the heavy metals (HM) contamination influence (Kumar et al., 2022; Damian et al., 2019; Samokhvalova, 2017; 2018) are an urgent, multifaceted and complex problems to solve today.

A special place among remediation activities is occupied by effective compositions of preparations, which contain biologically active chelated compounds of trace elements (TE) together with humates, which bind and reduce the chemical activity of various natures pollutants and prevent their migration from the soil to adjacent environments. Such specific properties of drugs chelate-humate compositions and complexes support their further use. Their ability to biodegrade pollutants (complete mineralization, minimal ecological «footprint») in the trophic chain - soil-plant-animal-human is important. TE is used by all living organisms in small quantities, however, their structural and functional role is significant due to the participation of TE in the transformation processes of substances and energy into biological (microorganisms, plants, animals, humans) and bioinert (soil, silt, weathering crusts; sea, river and lake water, etc.) systems. Each of the TEs performs strictly certain functions and cannot be replaced by another element. The use of TE is an important condition for increasing the production and quality of agrophytocenoses products, because the removal of nutrients (macroelements N, P, K; TE - Mo, Zn, Fe, Co, Mn, Cu, I, B, etc.) from the soil increases with the growth of plant productivity, the balance of nutrients is disturbed. Lack or excess of TE certain in plant products and feed can cause human and animal diseases. Therefore, TE is included in microfertilizers (inorganic compounds; frits; synthetic chelates and organic complexes; nanomicrofertilizers), which differ significantly in properties (physical state, chemical activity) and availability of the active substance for plants.

It was established (Bulygin et al., 1999), that from a biological point of view, ethylenediaminetetraacetic acid (EDTA) is a promising complexone for the creation of microfertilizers with the aim of soil introducing to replenish the lack of TE that affect the quality of agricultural products. However, until now, it is impossible to give an unequivocal answer to the question of which of the known complexones (EDTA, DTPA, OEDF, etc.) should be used to obtain biologically active TEs.

The main role belongs to the metal, and the complexion plays the role of a "vehicle" that ensures the delivery of the cation and its stability in the soil and applied nutrient solutions. Complexones determine the effectiveness of microfertilizer, which is determined by the degree of absorption of TE by plants.

In addition, the production of chelated microfertilizers is high-tech and knowledge-intensive. The tasks of elaborating new preparations containing TE chelates with the effects of fertilizer and stimulation of plant growth are being updated. In addition, the properties of complexones and complexonates based on them should be taken into account.

An important, complex and still unresolved issue for producers, as well as for consumers of chelated microfertilizers in Ukraine, is the degree of TE chelation, as an indicator of the predicted manifestation of microfertilizer effectiveness. In Europe, this indicator is fixed in the EU Directive 2003/2003 and is at least 80% (Regulation EC, 2003). There are no such regulatory documents in Ukraine.

Thus, the use of complexons and preparations based on them has a spectrum of application limitations, which should be taken into account both in the elaboration of chelated microfertilizers, chelated-humate preparations and in the practice of their use under background conditions and the influence of man-made pollution.

The presence of the listed imperfections requires the further elaboration of new highly effective, balanced in composition, complex preparations of fertilizing action, which are effective in the soil-plant system, in order to increase productivity under background conditions and improve its ecological condition under the pollution influence.

Exploring and exploiting soil improvers with a high adsorption capacity and low cost should be an effective method for overcoming the remediation problem HM pollution of soil-plant system.

The purpose of the research is to elaborate a method of obtaining a chelate-humate drug with a fertilizing-stimulating and remedial effect to improve the ecological state of the soil-plant system due to: (1) creating and using a new effective composition of the drug that ensures soil saturation with TE in a plant-available form, increasing the productivity of plants, including under conditions of pollution by heavy metals (HM); (2) simplification of obtaining and using the drug while saving resources.

MATERIALS AND METHODS

The method elaboration included expertise in conducting scientific researches, namely:

1. *Stage of informational and analytical research*. Analysis of methodical approaches to the elaboration of new chelate-humate preparations, including under the conditions of man-made pollution of HM; conducting a patent search (DSTU 3574 and DSTU 3575, 1998) and forming a working idea (hypothesis). Researched objects: available sources of scientific information (catalogues of periodicals, card files of articles and reviews using information and search systems, databases and data banks, the Internet), including databases of scientific and technical information on copyright objects that are patented in Ukraine and the countries of the post-Soviet space and EU, in context of the goal with an emphasis on the stages of known technical solutions, methods of obtaining them and fields of application. Research methods - methods of theoretical analysis, systematic approach.

2. *The stage of exploratory and field research.* Conducting exploratory researches in 2012 - 2014, microfield experiments (MFE) in the

natural-climatic zone of the forest-steppe (Sumy region) and steppe (Donetsk region) of Ukraine during 2014 - 2016, including taking into account soil contamination of the HM for the approval of a new Smaragd chelate-humate preparation (CGP). The experiments were carried out in accordance with the field experiment method (Dospekhov, 1985). The objects of the study are individual indicators of microbiological and enzymatic activity of different types of soils, the content of TE /HM (Cu, Co, Mn, Fe, Zn, Ni, Cr, Cd, Pb) mobile forms in the soil-plant system, the new Smaragd CGP, test plants (productivity, elemental composition).

The fertilizing-stimulating and remedial effect of Smaragd CGP using and the improvement of the soil-plant system ecological state was established by conducting MFE on the chernozem typical medium-loam of research ground within the Okhtyrka city, Sumy region; including under the influence of mono - and multi-metal Cd, Pb, Zn, Ni pollution. The test cultures - *Hordeum vulgare* L., *Lupinus angustifolius* L. The spectrum and levels of soil pollution of the HM correspond to the existing levels of pollution around PJSC "Sumikhimprom", where the excesses of the background content of Cd and Ni in the soil we established differs in 4 and 6 times, respectively; Pb and Zn - in 8 times.

Scheme of the experiment (12 variants, performed in triplicate): (1) Control; (2) Control + Smaragd CGP; (3) Cd soil pollution (4 background) + Smaragd CGP; (4) Cd soil pollution (4 backgrounds); (5) Pb soil pollution (8 backgrounds) + Smaragd CGP; (6) Pb soil pollution (8 backgrounds); (7) Zn soil pollution (8 backgrounds) + Smaragd CGP; (8) Zn soil pollution (8 backgrounds); (9) Ni soil pollution (6 backgrounds) + Smaragd CGP; (10) Ni soil pollution (6 backgrounds); (11) ∑HM pollution + Smaragd CGP; (12) ∑HM pollution.

Smaragd CGP was applied to the soil before sowing the test plants in a specified dose of 2.5-3 l/ha and 1.5-2 l/t - for seed treatment; 2.5-3 l/ha - for feeding plants in the phase of their active growth and development, with doubling of the drug applied doses for Cd, Pb, Ni, Zn multi-metal soil contamination.

Approbation of the Smaragd CGP was also carried out in field studies on chernozems ordinary of the Steppe zone of Ukraine (laboratory of soil fertility and soil protection technologies of the State Enterprise "Donetsk" NSC "IGA named after O.N. Sokolovsky"). Scheme of the field experiment (3 variants, performed in triplicate): (1) Control; (2) Reacom, 3 l /ha; (3) Smaragd CGP, 3 l /ha. The registered area of the trial plot is 25 m^2. The test crop - *Helianthus annuus* L. The effectiveness of foliar treatment of sunflower crops in the 12-leaf phase was studied.

The selection of soils samples from the arable (up to 20 cm) layer was carried out in accordance with the current regulatory documents - DSTU 4287:2004; DSTU ISO10381-6:2001. Selection of plant samples and their mineralization with the GOST 27262-87 and DSTU 7670:2014. All measurements were performed in triplicate.

3. *The stage of soils and plants samples chemical-analytical and laboratory researches*.

Determination of the orientation and intensity of individual microbiological and biochemical soil processes under the conditions of application of the new Smaragd CGP was carried out by analyzing soil samples in the Department of Soil Protection and the Sector of Soil Microbiology of the NSC "IGA named after O.N. Sokolovsky", according to the current regulatory documents and methodical base.

The following indicators of biological properties were determined in the soil samples: the number of microorganisms of the main ecological and trophic groups was determined by the method of microbiological sowing (by the deep method) of the soil suspension of the appropriate dilution on solid nutrient and liquid nutrient media (DSTU 7847:2015). Biochemical activity of soils was determined by the polyphenoloxidase activity (Karyagina and Mikhailovsky, 1986).

Atomic absorption determination of the TE/HM (Mn, Zn, Cd, Co, Ni, Fe, Pb, Cu, Cr) mobile forms content in soils and plants (DSTU 4770.1:2009 - DSTU 4770.9:2009; DSTU 7607:2015, DSTU 7831:2015, Methods of soil and plant analysis, 1999).

Determination of the cation-anion composition of water samples was carried out with current regulatory documents (DSTU 7525:2014; DSTU

7908:2015; DSTU 7909:2015; DSTU 7943:2015; DSTU 7944:2015; DSTU 7945:2015).

4. *Stage of chamber studies.* Establishing structural relations of soils biological and chemical properties based on the information estimation of the TE status of soils by means of expert evaluation of normative and reference documentation, conducting calculations of indicators of microbiological and enzymatic activity of the soil; statistical processing of the received data, including under the influence of HM man-made pollution; use of mathematical and statistical methods. Statistical processing of the received numerical data was carried out using the *Statistica* 10.0 software. Differences in average indicators were considered reliable at the significance level of $p<0.05$. *Microsoft Excel* 10.0 software was used to visualize the obtained data.

The assessment of the elemental status of the test plants was carried out in accordance with the current maximum permissible levels (MPL) of the chemical elements content in animal feed (Methodological guidelines, 1998). The assessment of the microelement status was carried out using the established background levels of TE content for the soils of natural and climatic zones of Ukraine (Fateev and Samokhvalova, 2012).

RESULTS AND DISCUSSIONS

Based on the results of long-term exploratory chemical-analytical and field researches in 2012-2016, we established the following: (1) conditions and components suitable for joint use, which promote the creation of new complex balanced fertilizer preparations that increase the synergistic effect of TE chelates (Fe, Mn, Zn, Cu, Co, Mo, B) using, where the chelating substance is oxyethylidenediphosphonic (OEDF), and humates in the established ratios of their concentrations, which ensure a high rate of their absorption by plants; (2) stability of the chelate-humate composition solution and the possibility of its further use in a wide range of component ratios, independence from water hardness; (3) increasing the efficiency of using chelated microfertilizers, in particular on carbonate and eroded soils; (4) fertilizing and stimulating effects of a wide range of chelate-humate composition ratios for the plants on soils of various types, the functioning of the soil-plant system as a whole, including the remedial effect under the conditions of mono- and polyelement technogenic soil pollution.

Due to the analysis of the existing patent documentation regarding to the subject of research, there have been established methods-analogues, which contain defined algorithms for obtaining and using new drugs. In particular, the well-known method of obtaining microfertilizers (Patent 64773 UA, 2004) involves the use of TE (Zn, Cu, Fe, Mn, Co, Mo, B) and complexone containing either carboxyl groups of one of the acids of the polyaminopolyacetic group (the most common ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), etc.) or phosphonic groups of one of the acids of the alkyd phosphonic group (the most common of them are OEDF, etc.) and the introduction of complexone of polybasic organic acid (in particular citric) in the ratio of complexons based on carboxyl or phosphonic groups of 50-80%, polybasic organic citric acid - 20-50 %, which increases the efficiency of microfertilizer nutrient absorption by plants several times compared to soluble mineral salts.

However, the use of artificial complexons of the III and IV hazard classes has limitations, including due to the high probability of their destruction under the influence of ultraviolet light, biodegradation and toxicity of the products of their transformation for plants and soil microorganisms (Williams, 1975; Dyatlova, 1988; Nanda, 2016), increased risks of soil pollution and groundwater, fixing of metals in the DNA of living organisms (Kasyanenko, 1989; Paston et al., 2016).

We established that the method of obtaining microfertilizer with a complex of biostimulators Yaramiks (Patent 75452 UA, 2012) involves obtaining a microfertilizer containing an aqueous solution of N, K, S salts and Fe, Mn, Cu, Zn, B, Mo, Mg, Co chelates; succinates, tartrates, citrates; lithium ions, complex lignosulfonates (LST), which are structurally similar to soil fulvic acids. However, given the established effectiveness of LST, their main drawback is the instability of the structure and functional groups, which requires the addition of compounds with oxidizing properties (nitrites, nitrates, etc.) and/or complexing agents - Cr (VI), Fe (III), Al (III), and/or organic compounds of alkaline reagents for LST stabilization, which leads to additional costs of resources and an increase in the cost of fertilizer.

We discovered the method of obtaining microfertilizer with a complex of biostimulators Nanomix (Patent 61566 UA, 2011) involves obtaining a microfertilizer with a complex of biostimulators, which contains an aqueous solution in a certain ratio of components - salts of N, K, S and Fe, Mn, Cu, Zn, B, Mo, Mg chelates; succinates, malates, tartrates, citrates, Co chelates and additional substances (growth stimulants - β-indolylacetic acid and/or β-indolylbutyric acid; polyvinyl alcohol, water); chelates based on OEDF, EDTA, ethylenediamine-disuccinic (EDDS), succinic, malic, tartaric and citric acids.

Obtaining microfertilizer is expensive due to the use of a wide nomenclature of specific fertilizer components, artificial complexes. Succinic, malic, tartaric, and citric acids are weak chelating agents for Zn, Cu, and Fe, with substitution in the soil solution and root zone of TE plants by calcium, which leads to a significant decrease in fertilizer efficiency and coagulation with humates.

In addition, based on the results of chemical-analytical studies, we established the incompatibility (coagulation) of TE preparations, in particular Nanomix fertilizers with alkali metal humates, which makes them limitedly suitable for use in compositions with this organic fertilizer and on alkaline soils.

The closest in composition and balance to the proposed complex chelate-humate preparation is the soluble organo-mineral fertilizer based on the organic fertilizer Biocycle-1 (Patent 86907 UA, 2009), which was obtained by extraction with alkaline reagents of the organic fertilizer Biocycle with a predominance of humates followed by the dissolution of organo-mineral components (urea, monosubstituted or disubstituted, or trisubstituted potassium phosphate, macroelements Mg and Ca; TE (Fe-Mn-Zn-Cu-Co) in the form of chelating salts, where the chelating substance is EDTA salt or its analogues, B and Mo, in the form of boric acid and sodium or ammonium molybdate; a mixture of polyethyleneglycols (PEG), plant growth regulators for processing seeds and vegetative mass of plants.

However, the analogue method is characterized by the high cost of obtaining fertilizer due to the need to involve modern equipment, significant doses of components for obtaining both new fertilizer and the organic-mineral fertilizer itself.

In addition, it is known that Mo and B in the composition of acids and salts are in the anionic form, which is characterized by a low level of assimilation by plants from the soil and does not form chelate complexes, comparable to cations of polyvalent metals. This creates difficulties in the fixation of Mo and B in water-soluble compounds, which are eliminated by the introduction of PEG, which are characterized by the transformation into a soluble state of difficult or insoluble components of the drug, increased absorption of substances, convenience and accuracy of dosing. Still, the main disadvantage of PEG solutions is the complexity of preparation and stabilization, large volume and the need to use special containers, etc. In addition, the organic component of the fertilizer is up to 70%, chelate - up to 3%, which leads to an increase in the risk of coagulation due to an increase in the content of the chelate component. Such an imbalance in the composition determines the introduction of more effective complexons in order to form of stable complexes with Mo, B.

Besides, the EDTA complex, which is used, is an unstable compound to the action of soil microorganisms and is capable of forming toxic hydrolysis products. EDTA-based TE chelates are restricted for use on soils with pH>7.5. For each element, stable compounds are formed only at certain pH values. The result of which is an increase in the risks of violation of ecological conditions in the root layer of the soil, a decrease in the biological and ecological safety of organo-mineral fertilizer, the efficiency of its use and the TE assimilation by plants.

Growth stimulators and regulators, as a component of the organic-mineral fertilizer based on Biocycle-1, belong to the 3rd and 4th class of danger; their specific effect on plants has not been sufficiently studied. The use of stimulators and growth regulators must be strictly regulated. According to the EU directive

2003/2003 of 13.10.2003, mandatory registration is provided for in the relevant state bodies separately for each country. Taking into account the fact that the majority of artificially synthesized specific growth stimulators are prohibited for application in agriculture, which significantly increases the risks of reducing the biological and ecological safety of fertilizer use.

In addition, the use of stimulators and growth regulators together with fertilizers requires highly qualified specialists, which increases the resource consumption of the method.

The elaborated algorithm of our proposed method includes the stage of obtaining and using Smaragd CGP:

1. *Preparation of Smaragd CGP* with fertilizing-stimulating and remedial action using a composition of chelate and humate components based on mixing their aqueous solutions. Soluble organo-mineral components containing TE in the form of chelated compounds using complexone, with additional introduction of Mo and B in the form of ammonium molybdate and boric acid to the chelated component of the preparation in the amount of 0.25-1.5 g/l and 10-20 g/l according to the established ratios of concentrations of other MEs chelates - Fe - 12-25 g/l; Mn - 15-25 g/l; Zn - 10-25 g/l; Cu - 5-10 g/l; Co - 0.15-2 g/l.

OEDF is used as a complexone in a stoichiometric amount with TE salts under constant stirring and pH control of the environment, followed by the introduction of a solution of known humate from organic raw materials of natural origin into the chelating component of the preparation, as an environmentally safe metal chelator. At the same time, the chelate and humate components of the composition interact in a ratio of 5:1-1:5, which makes it possible to obtain a water-soluble, liquid fertilizer Smaragd of the chelate-humate composition, balanced in terms of the TE content and humic substances.

As a chelating component of the drug, a solution of TE chelates used in the established ratios of active substances concentrations and OEDF at pH 5.4-7.5 for maximum assimilation of nutrients by plants.

In particular, to obtain 30 liters of the chelate component of the drug containing TE, aqueous solutions of their compounds are prepared by dissolving them in water in a volume sufficient for their complete dissolution, taking into account the established reference values of the product of their solubility. Seven containers are used, respectively, to dissolve iron chloride (III) ($FeCl_3x6H_2O$) - 1.92 kg; manganese sulfate ($MnSO_4xH_2O$) - 1.23 kg; zinc sulfate ($ZnSO_4x7H_2O$) - 1.75 kg, copper sulfate ($CuSO_4x5H_2O$) - 0.64 kg; cobalt sulfate ($CoSO_4x7H_2O$) - 0.15 kg; ammonium molybdate $(NH_4)_2MoO_4$ - 0.02 kg; boric acid (H_3BO_3) - 1.68 kg. Next, TE chelation is carried out by adding OEDF complexone in the form of powder, respectively 1.5 kg - 1.5 kg - 1.4 kg - 0.54 kg - 1.5 kg - 0.15 kg - 2.9 kg to each of the seven containers with ready-made aqueous solutions of TE with constant stirring and control of the pH of the medium with subsequent mixing of the obtained aqueous solutions of TE chelates into one.

By adding potassium hydroxide or potassium carbonate, the pH of the resulting solution of chelate compounds is adjusted to 4-7 units as needed. In this way, the chelating component of the Smaragd drug is obtained. The volume of its solution is brought up to 30 liters by adding water followed by filtering.

As a humate component of Smaragd drug, which contains low-molecular complexes with organic acids easily absorbed by plants, a solution of any available humate from organic raw materials of natural origin is used. For example, potassium humate extracted from peat with a humic acid content of 18.7 g/l; fulvic acid content - 8.8 g/l; pH - 9.7. In the solution of the chelate component of the drug in the amount of 30 liters, added a solution of potassium humate in the amount of 10 liters and we get the composition of the fertilizing Smaragd CGP with a high buffer capacity. In the combination of active agents of the proposed composition, water-soluble complexes of organic acids with multivalent metals are formed. The volume of the obtained concentrate is 40 liters; it is additionally filtered and packaged in a plastic container. The elaborated fertilizer Smaragd CGP is a homogeneous liquid of dark brown color with a pH of 6.5-7.5.

2. *Approbation of the new Smaragd CGP* by using the obtained preparation for pre-sowing treatment of seeds at a dose of 1.5-2 l/t and vegetative mass of plants at a dose of 2.5-3 l/ha with an increase in their productivity and adaptogenic properties, and introduction into the soil in a dose of 2.5-3 l/ha, and for multi-metal soil contamination with Cd, Pb, Ni, Zn by doubling the doses of the drug.

The effectiveness of the Smaragd CGP use to improve the ecological state of the soil-plant system by enhancing ecological functions (trophic, protective) was confirmed by an increase in the content of ecological-trophic groups - fungi, nitrogenfixers, and biochemical activity of the soil (Figures 1-3) according to the indicator, for example, polyphenol oxidase activity, productivity of test plants (Figures 4-6).

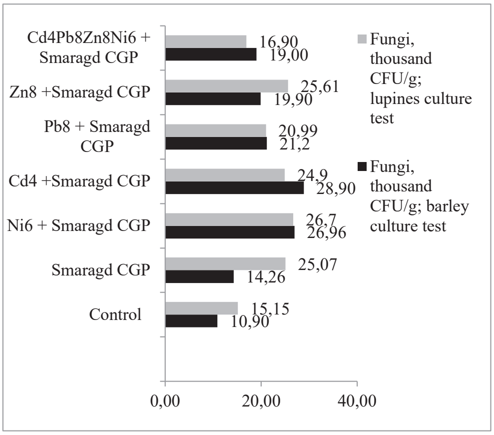


Figure 1. The influence of the Smaragd CGP use on the content of fungi in soil taking into account the test plants

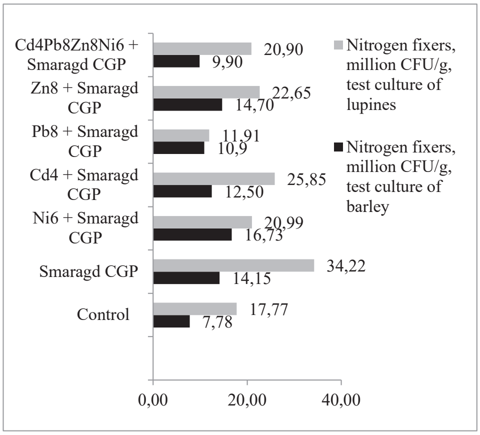


Figure 2. The influence of the Smaragd CGP use on the content of nitrogenfixers in soil taking into account the test plants

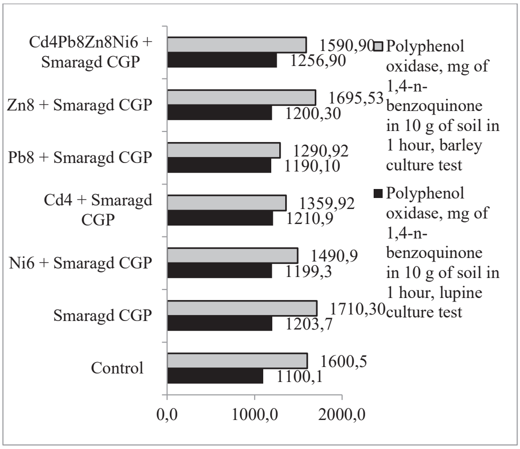


Figure 3. The influence of the Smaragd CGP on the biochemical activity of the soil taking into account the test plants

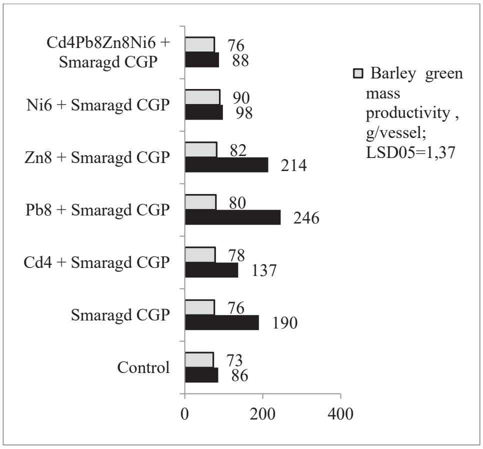


Figure 4. The influence of the Smaragd CGP on the productivity indicators of the green mass of the test plants

In particular, it was established that the Smaragd CGP introduction within the specified time period increased the productivity of grain, green mass and the root system of barley - by 21% and 4% and 2 times; lupine - the increase was 5 and 2.2 times, respectively, and by 46.6%. The Smaragd drug had a positive effect on the development of vegetative and generative organs of plants grown under soil monoelemental pollution with Cd, Pb, Zn, Ni (Figures 4-6). The effectiveness of the Smaragd drug was discovered and confirmed on Zn and Ni contaminated soil with the strengthening of the fertilizing and stimulating effect, increasing the adaptogenicity of plants and the productivity of green mass, grain and stimulating the development of the root system of test crops (Figures 4-6).

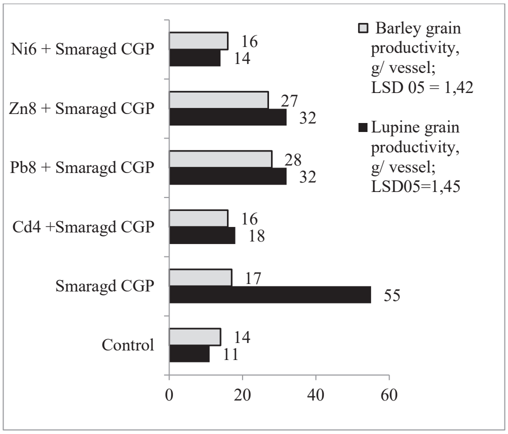


Figure 5. The influence of the Smaragd CGP on the productivity indicators of the grain of the test plants *Hordeum vulgare* L. and *Lupinus angustifolius* L.

[1]LSD $_{0.05}$ - Least Significant Difference at p = 0.05 in Figures 4-6.

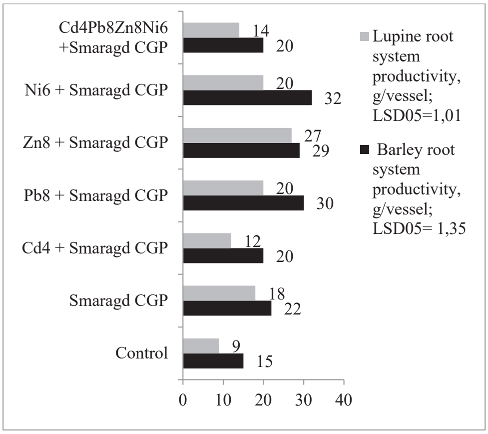


Figure 6. The influence of the Smaragd CGP on the roots system productivity of the *Hordeum vulgare* L. and *Lupinus angustifolius* L.

On MFE variants under the influence of soil HM multi-metal contamination, the use of the Smaragd CGP in the soil and foliar feeding of plants and pre-sowing treatment of seeds, a positive effect was obtained with an increase in the productivity of the green mass of lupine and barley by 2.3% and 4%, the root system - by 33% and 1.6 times, respectively. It was established that the saturation of the contaminated soil of TE with the introduction of Smaragd CHP leads to an increase in the content of mobile forms of Co, Cu (Figure 7), which ensures a decrease in the toxicity of Cd (Figure 8) in the soil-plant system, improvement of its ecological condition as a result of a certain optimization of the elemental status of the soil and the test plants - *Hordeum vulgare* L. and *Lupinus angustifolius*.

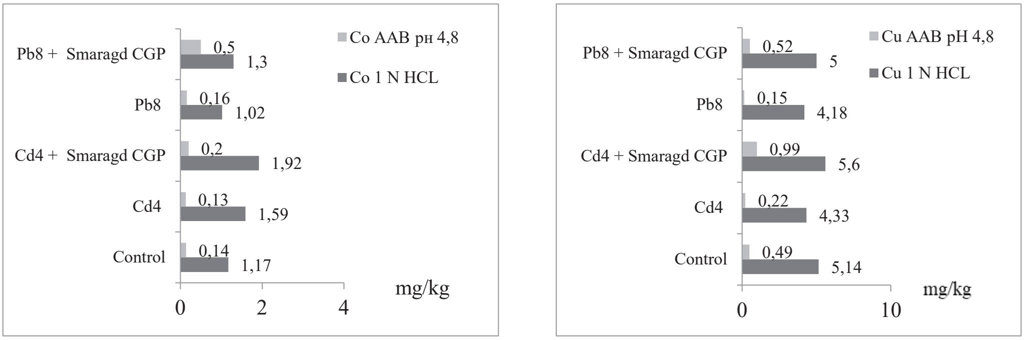
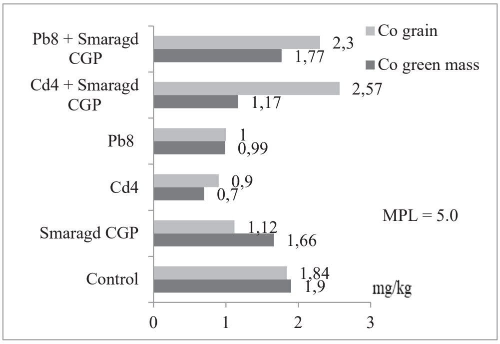


Figure. 7. The effect of the Smaragd drug use for the optimization of the elemental status of the soil

CHP Smaragd promotes the growth of ME content in barley test plants.

In particular, the accumulation of Co (Figure 8) and Fe increased 1.7 and 1.2 times in the vegetative mass and 2 times in barley grain for a decrease of 2 times or more in the content of the toxic metal Cd (Figure 8). A 1.7-fold increase in the content of Cu in barley grains was established, which proves the existing adaptogenic properties of the drug due to its influence on the optimization of the TE composition of test plants under conditions of HM soil contamination.



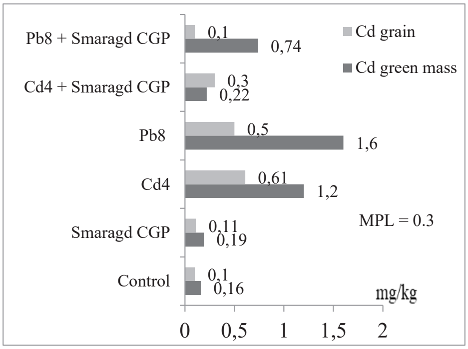


Figure. 8. The effect of the Smaragd drug use for the optimization of the elemental status of the test plants *Hordeum vulgare* L.

Thus, due to its fertilizing and growth-stimulating properties, Smaragd CGP contributes to the improvement of the ecological condition of plants with an increase in yield.

The Smaragd CGP does not have a limit on water hardness, compared to the existing ones. Data on the suitability of water of different hardness, with the established chemical analysis of the mineral composition, are given in Table 1. When using solutions of the components of the Smaragd CGP composition, the absence of coagulation due to excess calcium and magnesium in the water was recorded.

Approbation of the proposed Smaragd CGP in field studies on chernozems ordinary (DP DG "Donetsk") showed an increase in yield during foliar fertilization of sunflower (*Helianthus annuus* L.) in the phase of 12 pairs of leaves (Table 2), in contrast to the action of the drug Reakom, which forms insoluble complexes with calcium.

Table 1. Results of a standard chemical analysis of the mineral composition of different hardness water

Defined indicators	Current regulations according to DSaNPiN 2.2.4-171-10	Water sampling points for conducting experiments			
		Babai, Kharkiv region	Chuguiv, Kharkiv Region	Kharkiv, st. Otakara Yarosh, Sarzhyn Yar	Kharkiv, Florinka
pH	6.5-8.5	6.81	6.63	7.07	6.56
CO_2 mg/l	-	66.0	92.4	96.8	8.8
HCO_3^-, m-mol /l		7.2	7.8	3.9	10.2
Cl^- residual bound, m-mol /l	$\leq$1.2	6.8	1.8	4.0	1.6
SO_4^-, m-mol /l	$\leq$250	4.28	13.88	5.79	9.5
∑ anions, m-mol /l	-	18.28	23.48	13.69	21.3
∑ Ca^{2+}+ Mg^{2+}, m-mol /l	7-10	18.0	22.6	10.8	20.6
Ca^{2+}, m-mol /l	25-75	10.4	19.5	6.6	16.6
Mg^{2+}, m-mol /l	10-50	7.6	4.8	4.2	2.0
∑ Na^++ K^+, m-mol /l	-	0.28	0.88	2.89	0.7

Table 2. The effect of various drugs on the biological yield of the test plant in the conditions of the Steppe

Variant	Biological yield of sunflower (*Helianthus annuus* L.), soil - Chernozem ordinary (data table fragment)	
	t/ha	Increase, t/ha
Control	24.1	–
Reacom preparation, 3 l/ha	26.4	2.3
The Smaragd CGP, 3 l/ha	29.2	5.1
$LSD_{0.05}$	3.6	–

[1]LSD 0.05 - Least Significant Difference at p=0.05

Due to the proposed composition of Smaragd CGP, the occurrence of calcium coagulation is eliminated, both in soils and in plants; an increase in their effective action with OEDF during the period of agrophytocenoses cultures active vegetation is ensured, which was confirmed by an increase in the productivity and adaptogenicity of plants, taking into account the unfavorable conditions of increased carbonation and soil erosion (depletion of biogenic elements reserves in particular N, P and the content of mobile forms of TE in soils (Fe - 2 times, Zn - 3 times, Co and Cu - 5 times) according to the drug-induced resistance of plants to arid conditions during the test period.

Furthermore, according to the observations results of the meteorological indicators dynamics during field researches on the approbation of the Smaragd CGP action (Table 3), stable trends to an increase in the temperature background and a seasonal redistribution of the amount of precipitation with an increase in dry manifestations of the period of agrophytocenoses crops active vegetation were established (Pogromska, 2018). Our data confirmed the positive trend of regional changes in the number and intensity of extreme weather events (rainfall, average wind speed, intensity and frequency of storms, etc.) (Project Report, 2014).

The elaborated algorithms for the creation and use of the Smaragd CGP to improve the ecological state of the soil-plant system are protected by utility model patent 135145 UA, 2019. The elaboration refers to the methods and technologies of obtaining fertilizers and soil conditioners and their use, including in the case of man-made pollution of the soil-plant system with heavy metals.

Table 3. Meteorological indicators at the Sukha Balka agrometeorological station, Yasynuvatsky district, Donetsk region

Year	Air temperature, °C				Precipitation, mm				Soil surface temperature, °C			
	Months of the year											
	IУ	У	УI	УП	IУ	У	УI	УП	IУ	У	УI	УП
2010	10.6	18.4	24.1	26.5	32.0	95.0	59.0	40.5	14.4	22.7	31.3	31.6
2011	9.1	18.5	21.7	25.8	35.5	51.5	106.0	34.0	11.4	24.4	27.8	31.8
2012	14.6	20.4	23.0	25.6	26.0	66.5	88.0	17.5	17.7	25.9	29.4	30.8
2013	12.3	21.6	23.5	23.7	18.0	47.5	50.0	28.7	14.6	27.6	30.3	29.5
2014	10.4	19.5	19.9	24.3	27.0	85.5	95.5	76.5	13.9	22.4	23.8	27.6
2015	9.6	17.4	23.3	23.8	75.0	29.0	43.0	125.0	11.8	22.4	28.5	28.2
2016	15.1	19.6	25.9	29.9	40.0	87.0	39.5	8.5	14.4	18.6	24.0	28.1
2017	10.8	18.4	24.7	25.2	70.5	46.0	32.6	28.6	11.2	20.8	29.7	30.9
2018	14.5	20.7	24.5	25.5	20.8	25.9	40.7	88.4	15.0	22.6	27.5	27.0
1995 - 2005	10.8	17.5	21.5	24.3	43.8	41.6	91.4	64.2	12.9	21.6	27.4	30.2

A new method of improving the ecological state of the soil-plant system using the Smaragd CGP is advisable to use when growing plants in open and closed soil, hydroponics, under irrigation and fertigation conditions, pre-sowing incrustation of seed material, root and foliar feeding of plants in critical periods of their growth and development to increase quality and productivity levels and improve soil quality; as well as remediation of the soil-plant system HM contaminated, insufficient moisture on carbonate and eroded soils.

CONCLUSIONS

A new Smaragd CGP and an algorithm for its using on chernozem soils of the Forest-Steppe and Steppe zones of Ukraine were elaborated. Application of the algorithm ensures: (1) saturation of the soil with TE in a form available to plants; (2) improvement of adaptogenic properties with increased plant productivity; (3) improvement of the ecological state of the soil-plant system, including under conditions of soil pollution and arid conditions on carbonate and eroded soils due to the proposed effective composition of the drug, simplification of its preparation and use, and simultaneous resource conservation. The fertilizer is designed for pre-sowing seed treatment and plant feeding.

Distinctive properties and results of using the proposed Smaragd CGP, in comparison with known drugs, are as follows: (1) biological and ecological safety of the drug composition (quantitative and qualitative); (2) stability in a wide range of pH values of the environment, which ensures effective use of the drug on soils with a pH of 4.5-10 and increases its shelf life; (3) the preparation has no limitation on water hardness and is suitable for use in arid climatic conditions (high air and soil temperatures, minimization of precipitation); (4) the liquid form of the drug and the good solubility in water of the components of the proposed composition (metal complexes, gumatic component) enable consumers to obtain its working solutions in a wide range of chelated-gumatic ratios in the field in the necessary doses depending on the composition of the soil and the needs of the plants with simultaneous simplification of transportation and cheapening of its use; (5) the prolonged action of the drug, which is ensured due to the probable gradual release of TE from the drug regarding to the difference in the stability constants of metal chelates and the exchange capacity of fulvic and humic acids, as well as different solubility of complexes with a certain excess of OEDF complexone; (6) fertilizing and stimulating effect with the possibility of reducing the rates of introduction of components in terms of the active substance for its preparation and the rates of its application under plants and in the soil; (7) the remedial effect, ensuring the strengthening of the ecological (in particular, trophic, protective) functions of the soil and the improvement of the ecological state of the soil-plant system, including under the conditions of man-made HM pollution, arid conditions and on carbonate and eroded soils; (8) activation of growth processes of plants of various species and growth of their yield due to the mobilizing action of a natural components complex preparation.

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CURRENT APPROACHES TO CARBON MANAGEMENT FOR INCREASING ITS BUDGET IN SOILS

Yevhen SKRYLNYK, Kateryna ARTEMIEVA, Iryna KHYZHNIAK

"Institute for Soil Science and Agrochemistry Research named after O.N. Sokolovsky",
4 Chaykovska Street, Kharkiv, Ukraine

Corresponding author email: irinamikaella@gmail.com

Abstract

It is shown the average humus content in agricultural soils of different soil and climate zones of Ukraine. It is emphasized that today there is a positive trend in the humus content of soils in comparison with the data of previous years. The existing resources of organic raw materials for replenishment of organic carbon reserves in the soils of different zones of Ukraine have been analyzed. The characteristics of potential resources of organic raw materials of natural origin and organic waste from the standpoint of humus formation are given. Approaches to the management of organic materials to increase the efficiency of accumulation of humus in soils are proposed. In a model experiment, it is proved that humus of alluvial-meadow soils is easy to be mineralized in comparison with chernozem soils. Taking into account the peculiarities of meadow soil formation, approaches to carbon budget conservation in these environmentally sensitive soils are proposed.

Key words: *soil organic carbon, carbon budget conservation.*

INTRODUCTION

Soil organic carbon (SOC) is essential for soil fertility, for its ecosystem functions, especially for food production. SOC is one of the world's climate change factors because of excessive carbon dioxide emission from soils affected by imbalance of humification-mineralization processes of soil organic matter in favour of the last ones, which are due to current intensive and irrational soil use. This unqualified assertion is in the focus of fundamental and applied research both of national and foreign scientific experience (Kell, 2011; Xiao, 2015; Crowther et al., 2016).

According to the "World Soil Resources" edition (FAO, 2015), organic carbon budget of agricultural soils is 30 Gt in the USA, 26-28 Gt in China, 17-18 Gt in Russia and from 2-3 to 4-5 Gt in Ukraine (1 Gt is 1 bln tones). However, this huge SOC budget could be lost easily as a result of increased and unreasonable anthropogenic influence on natural equilibrium of soil-formation processes as well as a wide range of natural factors.

Indeed, during 1850-2005 years it was lost 74 ± 18 Pg of SOC due to erosion, 79-85% of which are on agricultural lands and pastures (Naipal et al, 2018). According to Regional soil change assessment in Europe and Eurasia (FAO, 2015), humus content is being lost on 23-70% of agricultural land areas and it was expectedly proved (Lal, 2010) that agricultural soils contain SOC by 25-75% less than their analogues but on natural ecosystems.

Total emissions of greenhouse gases released into the atmosphere on agricultural land in 2019 amounted to 10.7 billion tons of carbon dioxide equivalent (Gt CO_2 eq), a decrease of 2 percent, or 0.2 Gt CO_2 eq compared with 2000 (FAO, 2021).

Besides, according to the data of Maun-Loan laboratory, the concentration of carbon dioxide in earth's atmosphere went beyond 415 ppm grade at the date of the 11th of May, 2019 (Dockrill, 2019).

That is why soil carbon circle and sequestration management is a key link in solving such world's actual problems as combating soil degradation and climate change mitigation (FAO, 2017).

Development and implementation of methods for SOC sustainable management are extremely important for soil protection in Ukraine. Ukraine has joint the UNO Convention to Combat Desertification, where it adopted a voluntary national commitment to increase the organic carbon content in soils at least by 0.1 % by 2030. Increasing SOC budget will contribute to the improvement of soil ecological properties, including preservation and restoration of soil biota, optimization of nutrient and moisture regime thereby increasing the long-term resistance of arable soils to degradation under the influence of anthropogenic factors. Therefore, the relevance of the issues disclosed in this article does not raise any doubts.

MATERIALS AND METHODS

The research was conducted in certified laboratories: laboratory of organic fertilizers and humus (Certificate of compliance of the measurement system with the requirements of DSTU ISO 10012:2005, No. 01-0083/2020) and laboratory of instrumental soil research methods, standardization and metrology (Certificate of compliance of the measurement system with the requirements of DSTU ISO 10012:2005, No. 01-0083/2020) in accordance with current standards of Ukraine (DSTU).

During two tours of soil surveys (2006-2010 and 2011-2015), soil samples of different soil types were taken from the depth of 0-30 sm for laboratory analysis.

Organic carbon content in studied soils was determined by Tyurin method based on dichromate oxidation. Organic carbon content then was recalculated into humus using the mean coefficient (1.724). Humus composition was determined by Tyurin method according to Ponomareva - Plotnikova procedure (Ponomareva & Plotnikova, 1980) where different organic matter groups were separated into humic acids (HA) and fulvic acids (FA) with further calculation the HA/FA value.

All measurements were performed in triplicate. Statistical analysis of variance was performed using Statistica 10 software.

Generalization of main characteristics of available organic fertilizers and local raw materials of different origins was carried out on the basis of the assessment of statistical reports of the laboratory of organic fertilizers and humus.

Soil samples were also taken from the meadow soils (0-30 cm depth) on the middle part of floodplains of the Left-bank Forest-steppe zone of Ukraine for analysis and laboratory-model experiments.

A laboratory-model experiment was conducted for establishing the susceptibility of meadow soils humus to mineralization processes. For experiment, there were taken loess heavy loamy, chernozem podzolized heavy loamy, meadow heavy loamy soils from different floodplains and meadow residual saline light loamy soil. The experiment involved composting soil samples in glass vessels in the thermostat at a temperature of 20-22°C and humidity of 60% of the total moisture content.

Soil samples without composting were used for control. The mass of air-dry soil in each vessel was 500 g. The study was five times repeated. The experiment lasted 9 months. At the end of every three months period it was determined the amount of accumulated mineral nitrogen in composted soil samples (according to DSTU 4729:2007).

RESULTS AND DISCUSSIONS

Anthropogenic influence and interference into soil natural processes disrupts the process of humus accumulation, which leads to a ratio changes in synthesis and mineralization of organic matter in soil. Processes of dehumidification cause deterioration of agronomic properties of the soil and reducing its fertility, which may affect functioning of the biosphere as a whole.

According to the latest tour of lands agrochemical certification (2011-2015), agricultural soils of Ukraine are characterized mainly by high humus content (3.07%). This reflects the positive trend of soil humus content compared to the data of the previous tour of surveys (2006-2010), which revealed average humus content of 3.05%. This trend is due to the increase in the supplying of organic matter to the soil, mainly due to plant residues. For example, over the last five years, 2.5 to 4.8 t ha^{-1} of straw and 11.6 to 16.6 t ha^{-1} of green manure have been applied annually. In terms of soil and climatic zones, the most significant increase in humus content in soils was observed in the Polessye zone (4% compared to 2006-2010), the least was in the Forest-Steppe zone - 0.6 % and Steppe zone - 0.1 % (Figures 1-3).

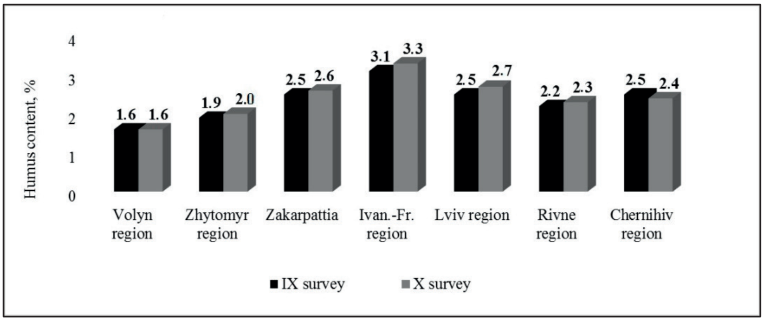


Figure 1. Humus content in soils of the Polessye zone of Ukraine [IX (2006-2010) and X (2011-2015) tour of the survey], %

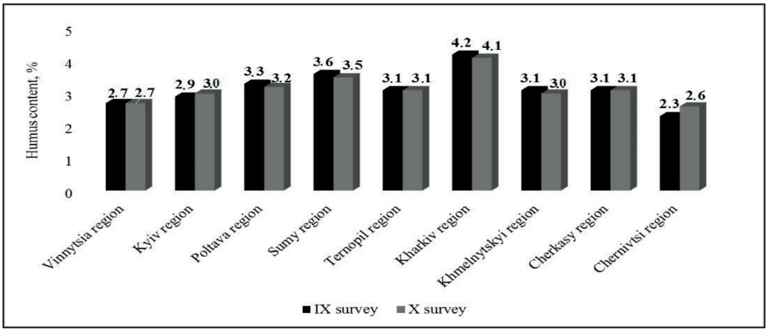


Figure 2. Humus content in soils of the Forest-Steppe zone of Ukraine [IX (2006-2010) and X (2011-2015) tour of the survey], %

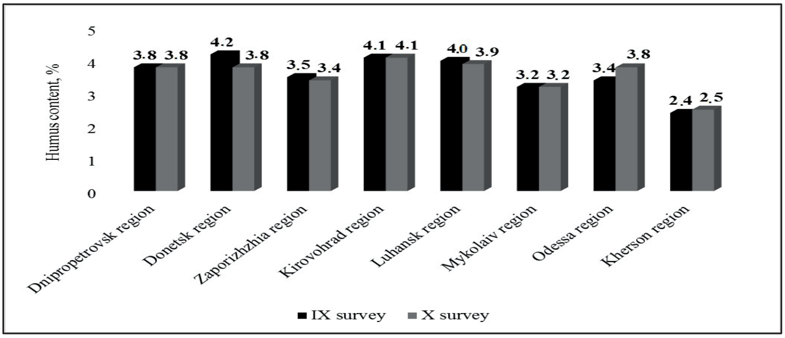


Figure 3. Humus content in soils of the Steppe zone of Ukraine [IX (2006-2010) and X (2011-2015) tour of the survey], %

Preventing soil degradation, reducing the risk of environmental disturbances during crops growth as well as stabilizing soil carbon stocks necessitates the use of organic raw materials, such as organic fertilizers, crop residues and local raw materials with maximum fertilizing and reclamation effect (Skrylnyk et al., 2019; 2020).

According to statistic data, the production and use of organic fertilizers have decreased almost 16 times during the last 30 years. Currently, the use of organic fertilizers ranges from 0.13 to 1.20 t ha^{-1} in different zones of Ukraine.

For the last five years the largest amount of organic fertilizers was applied in the Polessye zone (from 0.8 to 1.2 t ha^{-1}), the smallest - in the Steppe zone (from 0.13 to 0.25 t ha^{-1}). Organic fertilizers are mainly applied to fodder crops, the least - to cereals (except corn). As of 2020, only 1.9 % (0.8 million ha) of manure was applied.

The total annual output of manure in farms of all categories was 106.2 million tons in 2020. As for calculations of 2020, the use of all manure and poultry manure (in terms of a litter manure) provided fertilization of agricultural land with traditional organic fertilizers at a dose per dry matter in the Polessye region - 1.9 t ha^{-1}, in the Forest-Steppe zone - 1.5 t ha^{-1} and in the Steppe zone - 0.5 t ha^{-1}.

Currently, the measures that promote greater inflow of organic matter into the soil include the use of plant residues (Skrylnyk et al., 2019).

According to 2020, in case of plowing the by-products of the main agricultural crops, their application is possible in the Polessye zone at a dose of 4.9 t ha^{-1}, Forest-Steppe - 6.4 t ha^{-1}, in the Steppe zone - 3.4 t ha^{-1}, which in terms of litter manure is 2.8 t, 3.2 t and 1.9 t of dry matter per hectare of sown area in zones of Ukraine, respectively.

Thus, considering the available output of manure and by-products of the main crops in Ukraine, the estimated possible application of organic fertilizers, is 4.7 t of dry matter equivalent to litter manure per 1 ha of sown area for the Polessye zone, 5.9 t ha^{-1} for Forest-Steppe and 2.5 t ha^{-1} for the Steppe zone, while the norm for ensuring a deficit-free balance of humus, depending on the soil-climatic zone, should be from 8 to 14 t ha^{-1}.

The calculation of organic fertilizers doses is based on the indicator of the total nitrogen content. The greatest efficiency of organic fertilizers is provided when applying them in the recommended application norms. The increase in organic fertilizer rates causes, on the one hand, a significant decrease (by 1.5-2 times) in cost recovery and profitability, and, on the other hand, unwanted ecological consequences such as environmental pollution and deterioration of the ameliorating effect of organic fertilizers.

There is necessity to control the quantity and quality of carbon compounds entering the soil for regulation the humus state of arable soils.

Proving the role of organic fertilizers as humus-formers requires data of their chemical composition on total content of nutrients and the composition of their organic matter as well.

A specific feature of the chemical composition of most organic materials compared to plant residues and by-products is that they contain "finished" humic acids (Burdon, 2011; Skrylnyk et al., 2016; Drichko et al., 2013). The quantity of these compounds is different and depends, mainly, on their content in the organic filler, and on the intensity of humification processes during biocomposting of raw materials. In addition to humic acids, organic fertilizers contain various components that are easily and difficult to decompose. For example, manure on straw bedding contains a small amount of humic acids and an increased amount of carbohydrates and lignin.

During storage, the organic matter of manure changes significantly (Table 1). As an example, the content of carbon in mold is 5 and 3 times less than that of fresh and rotted manure, respectively.

Table 1. Organic matter composition of cattle manure of different storage period

Sample	C_{total}, %	Alkaline-pyrophosphate extract				Alkaline (NaOH) extract			
		C_{total}	C_{HA}	C_{FA}	C_{HA} of C_{total}	C_{total}	C_{HA}	C_{FA}	C_{HA} of C_{total}
		%							
Fresh	34.8	2.9	1.2	1.7	41.4	5.9	4.0	1.9	67.8
Half-rotted	28.3	1.9	1.3	0.6	68.4	5.2	3.4	1.8	65.4
Rotted	22.8	2.1	1.1	1.0	52.4	3.9	2.9	1.0	74.4
Mold	7.6	0.7	0.3	0.4	42.9	1.0	0.9	0.1	90.0

During the composting, the qualitative composition of organic matter of manure is being transformed as well, the content of humic and fulvic acids decreases.

Fresh manure consists mainly of fulvic acids (about 60% of the total carbon of the alkaline-pyrophosphate extract). At the half-rotted stage, the content of humic acids twice exceeds fulvic acids in the composition of humic compounds, i.e., alkaline-pyrophosphate solution extracts more than 68% of humic acids. At above mentioned stage of manure decomposition alkaline solution also extracts up to 65% of humic acids, and already at the stage of rotting - more than 74%.

Due to the shortage of traditional organic fertilizers, the role of attracting local raw materials of various origins to replenish organic carbon reserves is growing.
Natural raw materials (sapropel, peat, lignite, leonardite, biochar), organic wastes from processing industry and utilities (molasses, pulp, lignin, organic raw materials from biogas system, sewage sludge), livestock and poultry farming (solid and liquid manure, droppings), vegetable raw materials (green manure, by-products) are alternative sources of organic matter in soil and raw materials for production organic and organo-mineral fertilizers (Figure 4) (Brassard et al., 2019; Skrylnyk et al., 2020, 2021; Hetmanenko et al., 2021; Seadi et al., 2012).

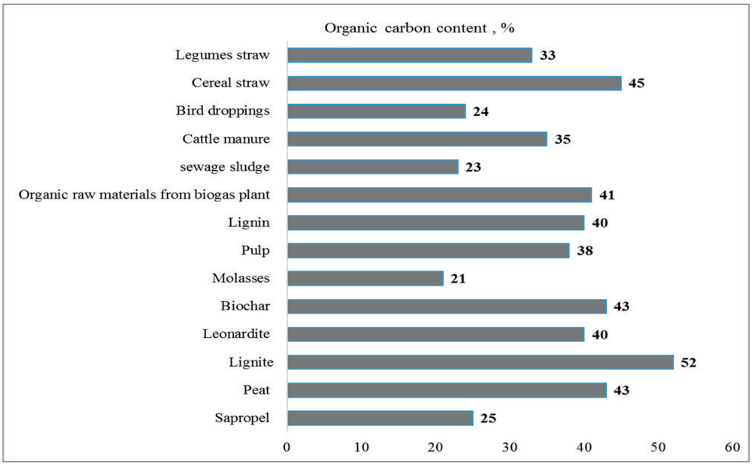
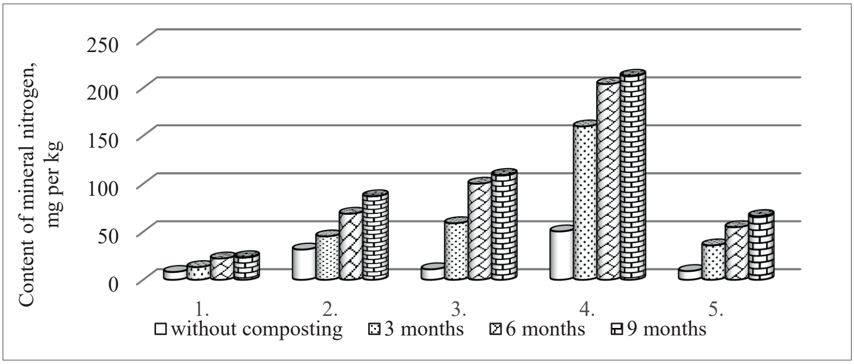


Figure 4. The average content of organic carbon in organic raw materials of various origins

Production of crop by-products in Ukraine exceeds 80 million tons per year. Predictably, the possible output of the crop by-products is for grain crops - 22.5 mln t (75% of the total output for fertilizer); corn per grain - 46.0 mln t; sunflower - 23.6 mln t; soy - 2.7 mln t; vegetables - 6.8 mln t. On average, up to 400 kg of carbon, 8.5 kg of nitrogen, 3.8 kg of phosphorus, 13 kg of potassium and microelements are added to the soil from 1 t of straw. During the decomposition of the cereal crops residues or their straw in the soil without the addition of nitrogen from mineral or organic fertilizers, the humification coefficient does not exceed 8-10%. The application rate of nitrogen fertilizers should be differentiated, depending on the type and amount of plant residues left after harvesting. This approach to fertilizing will not only improve the humus state of the soil, but also reduce the unproductive loss of nitrogen.
The importance of using crop residues as organic fertilizers is also justified from an economic side, because it requires minimal additional costs, since it is simultaneous with harvesting.
As for hydromorphic soils, they don't require additional management for increasing humus content, because naturally it is on a high level (at times up to 20%), but due to their natural ecological specificity it could be easy mineralized when these soils are overused. According to the results of model experiment with composting soil samples of various genesis, at the end of the experiment the content of nitrogen in hydromorphic meadow soils increased approximately 10 times, compared to variant without composting (Figure 5) and it was the highest amount among all variants of the experiment. On the other hand, after 9 months of composting the automorphic soil and loess, the content of mineral nitrogen increased only 3 times, compared to variant without composting. It shows that humus of automorphic soils is more stable to mineralization in contrast to hydromorphic soils.
Thus, overuse of hydromorphic soils may lead to the strengthening of mineralization processes, which can cause eutrophication of groundwater and nearest water sources with nitrates and

increase the emission of greenhouse gases from these soils.

In this regard, soil carbon management of hydromorphic soils should be mainly focused on preservation available soil carbon budget and prevention its further loses. Only implementtation of sustainable management on these soils will help to achieve above-mentioned aims.



1 - Loess heavy loamy.
2 - Chernozem podzolized heavy loamy.
3 - Meadow heavy loamy soil.
4 - Meadow heavy loamy soil.
5 - Meadow light loamy soil.

Figure 5. Content of mineral nitrogen in different soils after 9 months composting

Sustainable management of hydromorphic soils means keeping differentiated approach to the use of meadow soils, introduction of cultural hay and pasture areas with regulated regime of use, implementation of contour and phytoameliorative system (selection of meadow grasses mixes, most adapted to specific soil and hydrological conditions) and cultivation of energy crops (Khyzhniak, 2020).

The use of these soils as arable lands requires strict adherence to scientifically based recommendations, balanced actions and, above all, the use of soil-protecting agricultural methods and technologies.

CONCLUSIONS

There is a deficit of available manure and by-products of the main agricultural crops in various soil and climatic zones of Ukraine and even their maximum application into the soil does not ensure the recommended zonal norms. The possible dose of applying organic fertilizers, such as entire amount of manure, by-products of cereal and legume crops with addition the 40% of the annual amount of cereal straw, reaches on average only 2 t per ha in Ukraine (equivalent to the dry matter of litter manure per hectare of sown area).

Due to the shortage of traditional organic fertilizers, the role of raw resources of various origins (natural raw materials, organic waste from the processing industry and utilities, animal husbandry and poultry farming, plant raw materials, etc.) is significantly increasing for the replenishment of organic carbon reserves in the soils of Ukraine.

Organic waste is proposed to consider primarily as a source of carbon-containing compounds and plant nutrients after appropriate technological decisions have been made.

Systematic use of organic materials in agriculture will lead to reducing the imbalance of biogeochemical carbon cycles and increasing the stability of agrocenoses.

In order to conduct scientifically based optimization of the humus state of soils, it is necessary to control the quality of organic fertilizers, taking into account the characteristics of the organic component, which is an important factor influencing the processes of mineralization and humification of carbon in the soil.

A balanced zonal scientific and methodical approach to the management of organic raw materials in the context of increasing the carbon content in the soil will contribute to preserving the fertility of the soils of Ukraine, rational nature management and protection of environment.

For the preservation of huge budget of organic carbon in hydromorphic soils there should be justified, controlled and regulated agricultural activity with the implementation of sustainable management practices.

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SUPPLY AND DISTRIBUTION DEGREE OF SOME MACRONUTRIENTS IN SOILS POLLUTED WITH HEAVY METALS NEARBY THE CITY OF COPȘA MICĂ

Ioana Andra VLAD[1], Győző GOJI [2], Szilard BARTHA[1]

[1]University of Oradea, 26 General Magheru Street, Oradea, Romania
[2]Tehnological High School "Stefan Manciulea", Blaj, Romania

Corresponding author email: ggyozo2000@yahoo.com,

Abstract

Nitrogen (N), potassium (K), and phosphorus (P) are macronutrients that are paramount for plant physiology. The aim of this research is to evaluate the content and distribution of total N, and mobile K and P in soils historically polluted in the Copșa Mică area. We also calculated an average content of mobile P and K, highlighting the variation of the C/N ratio in the shallow depth layers of the soils within the sample surfaces. The negative impact on the soils of the main polluter of the Copșa Mică area is corroborated with a low total N content, minimal K concentration at the first depth layer, following a trend characterised by extremely low concentrations at the depth of 10-15 cm to extremely low to very low concentrations at the depth of 30-35 cm. The same trend is also noticed in the content of mobile P, the lower concentration classes being favoured in terms of average P content.

Key words: *soil nutritional status, total N, mobile K and P, polluted area.*

INTRODUCTION

Carbon (C), Nitrogen (N), Phosphorus (P), and Sulphur (S) are vital macronutrients required in vital doses for the life cycle of plants. The main source in soil nutrients generationis the decomposition of litter and humus followedbytheirfermentation, and fossil fuel extraction provides a 1000-fold surplus of C and N and 10-fold for P over the rate of natural rock degradation processes (Sposito, 2008). The biogeochemical cycles of these elements are impacted in areas facing high anthropogenic pressure or in the case of soil supplementation with exogenous fertilizers (Robertson and Vitousek, 2009). Pollutants, by means of interacting with soil components, but also microorganisms and plant root cells modulate the dynamics and bioavailability of nutrients (Saha et al., 2017; Aslam et al., 2014). Kozlov and Zvereva (2007a) and Zverev (2009), showed that the toxicity of polluting heavy metals is the main factor containing the natural regeneration of areas heavily destroyed by pollution, the research highlighting a significant decrease in the thickness of the organic soil layer by an average of 40% compared to control surfaces nearby non-ferrous smelters. In case of the reduction and total disappearance of the topsoil, Hein and van Ierland (2006) noticed a complete regression and a full cessation of forest regeneration.

Carbon in soil

Humus is the main source of C, N, S, and P for plants (Sposito, 2008; van de Wal and de Boer, 2017). The term of humus underwent several approaches synonyms and interpretations in the dedicated literature (Tan, 2011; Yarow, 2015; Paul, 2016). Thus, several researchers consider humus associated with the extractable fraction of organic matter synonymous with humic substances or soil organic matter (Whitehead and Tinsley, 1963; Kumada, 1987; Haider, 1994). The increase in organic matter is largely due to soil biodiversity, root exudates and microbiological nitrogen fixation (Safari and Rashidi, 2012; Thiele-Bruhn et al., 2012). Soil decomposition, washing, erosion, and runoff phenomena depletes the carbon from soil. Significant amounts of heavy metals i.e. Al, Fe, Cu, Zn, Mn, Hg, Cd from acidic soils are inactivated by chelation with humic acids, thus diminishing the solubility, bioavailability, and toxicity thereof (Zhang et al., 2009; Shan, 2016; Mikkelsen et al., 2020).

N dynamics in soil

Nitrogen is the macronutrient of plant growth and reproduction (Osman, 2013). Processes that are part of the N cycle are dry or wet atmospheric deposition, biological fixation such as degradation/decomposition.

In soil, N is represented by organic forms susceptible to slow mineralization into bioavailable forms (Stevenson, 1982b), and minerals (soluble NO_2^-, NO_3^-, exchangeable/non-exchangeable NH_4^+). Mineral N is less than 5% of total N, being used by plants but potentially harmful to the environment. Târziu (2006), showed that in the case of forest soils more than 50% of the total N is found in the first 20 cm of the soil profile. N leaves the soil in gaseous form (N_2, N_2O_5, and NH_3) and through soil leaching due to high mobility (Qafoku et al., 2000; Sposito, 2008). Sedimentary rocks, the activity of microorganisms and symbiotic mycorrhizae along with anthropogenic addition can increase N content in soil (Vitousek and Howarth, 2007; Morford et al., 2011).

The C/N ratio quantifies the relative content of N in organic matter, regulating the balance between N bioavailable to plants and N, which is leached or denitrified (Qafoku et al., 2000). Generally, C/N ratio in soil ranges from 10.5 to 31.5, with values between 10-12 in uncultivated soils that decrease with depth. Forest soils have a higher C/N ratio i.e. from 20 to 30, much higher ratios (i.e. $\geq$150) being characteristic in case of forest soils with acidic litter. Wet acidic deposits with NO_x or SO_x content can be beneficial for the soil N budget, although they are quantitatively lower than the amount of 100-300 kg N/ha/year required for agricultural crops, or even lower amounts needed for forest ecosystems. The harmful action of acid deposition manifests through foliar lesions, poorer resistance to pathogenic or entomological aggressions.

Characteristics of P in soil

After N, P is the second limiting element of terrestrial production (Kvakic et al., 2018). The total concentration of P in the soil varies between 50-3,000 mg/kg, the inorganic form reaching 50-70% while the share of available P is only 2.5-5%. Due to the current status Cox (2001) and Prasad et al. (2016) suggest for quantitative studies to determine the P level available in soil. Guertal (1991) noticed a stratification of P concentration values in the case of unploughed soils, at the depth of 0-2 cm the level of extractable P being extremely high, which can predict the risk of losing significant amounts of P. The P load of the soil may increase through the mineralization of organic P from plant residues, the secretion of phosphatases by plants, the activity of soluble microorganisms (Zaidi et al., 2009; Bhattacharyya and Jha, 2012) or inorganic P immobilization in microbial biomass (van der Heijden et al., 2015). Losses of P through leaching, erosion or gaseous emissions are insignificant (Sposito, 2008). Soluble compounds with P manifest a high reactivity and low mobility triggering the retention of Cu, Zn in the soil (Ullah et al., 2017; Sohail, 2019).

Presence of K in soil

K is the macronutrient that plays an important role in various plant metabolic processes (Rehm and Schmitt, 2002). According to Sparks and Huang (1985), K in soil represents a dynamic equilibrium between the exchangeable form, in solution, as mineral (from where it can be mobilized by K-solubilizing bacteria), and non-exchangeable form. Foth and Ellis (1997) showed that 98% of total soil K is unavailable to plants, and extractable-available K represents only 0.1-2%. The amount of K available to plants is in exchangeable form and K^+ from the soil solution, and depends on K content, the rhizospheric microbiome, mycorrhizal associations and organic root exudates (Sheng et al., 2003; Yousefi et al., 2011; Gundala et al., 2013), cation exchange capacity (CEC) or the level of other cations. K leaves the soil through leaching and erosion. The pH values in the range of 6.5-7.5 are optimal for the bioavailability of N, P, K. (Hochmuth et al., 2014).

The impact of multi-decadal pollution on the edaphotopes in the Copșa Mică area

The quality of the soils around the city of Copșa Mică in terms of historical pollution impact (i.e. over 70 years) was targeted by several scientific research works conducted especially by Răuță et al. (1987), Comănescu et al. (2010), Szanto et al. (2012). Barbu (2006) and Ianculescu et al. (2009), noted that forestry is most affected by pollution. The area where the experimental site was placed largely coincides with the territory of maximum pollution of approx. 7,900 ha, a fact highlighted by Alexa et al. (2004), Szanto et al. (2012). In this context Alexa et al. (2004) noticed the reduction of the supply of nutrients and the damage to the soil humification as obvious markers of pollutants impact on local edaphotopes. Regarding the heavy metals with toxic potential (i.e. Pb, Cd and Zn), Nedelescu et al. (2017), Alexa et al. (2004), Muntean et al. (2010) found concentrations that far exceed the maximum limits allowed for the upper horizon of the soil around the town of CopșaMică, but also for the Micăsasa commune.

The point sources of pollution within the area were the following: Carbosin S.A. companywhich activity was the production of blackcarbon through the chemicalization of methane until 1993, which made its mark on an area of approx. 20 km long and 6 km wide (Iordache, 2020) with polluters traces that are reminiscent even today and Sometra S.A. company which suspended its major activity since 2009. Some species used in the ecological restoration of the area between 1988-2008 such as: black locust (*Robiniapseudacacia* L.), Russian olive (*Eleagnus angustifolia* L.), desert false indigo (*Amorpha fruticosa* L.) or the white sea buckthorn (*Hippophaë rhamnoides* L.) aimed at improving the quality of soils through their ability to fix atmospheric N_2 (Alexa et al., 2004).

MATERIALS AND METHODS

In a holistic assessment of soil health, certain standard nutrient tests were put forward by various authors (Ashrad and Martin, 2002; Bajracharya et al., 2007; Fine et al., 2017; Frost et al., 2019) which subsequently have been widely tested in the US and India. These tests include, among others the determination of organic matter, P and extractable (bioavailable) K of soils. Determination of extractable nutrient values provides clear indications of soil capacity to support plants and one can find first those threshold or critical values and secondarily the environmental impact of some soil compounds (Dalal and Moloney, 2000).

The soil sampling took place between November 15 and December 4, 2009, in compliance with the rules for collecting soil samples issued by the National Research-Development Institute for Pedology, Agrochemistry and Environmental Protection - ICPA Bucharest ([xxx], 1981). The experimental site includes 13 sample plots(SPs) as follows: SP1 (Management unit - U.P.1 Veseuș/ Forestry District O.S.Aiud); SP 2 (Management unit U.P. II Micăsasa/Forest districtO.S. Mediaș), SP 6, 7East, 7West, 8 9, (Management unit U.P.III Târnava/ Forestry district O.S. Mediaș); SP 10 (Management unit U.P.VII Moșna/Forestry district O.S. Mediaș); SP 12, 13, 14, 15, and 16 (Management unit U.P.I Șeica Mică/Forest district O.S. Mediaș). For the comparison of the analytical values, a control surface SP1 protected from intense pollution was also required, and the site is located west to the city of Blaj, at 26.36 km away from the source of pollution. Sampling was carried out outside cold season and minimal plant physiological activity (Pennock et al., 2006), with maximum accuracy to prevent mixing soil layers from different depths.

Elemental samples were taken from the sample surfaces from four (4) different sampling points and mixed to obtain an average sample. Through the point-centered quarter (PCQ) method, this average sample was halved until obtaining the laboratory samples subject to analytical determinations (Dean, 2022). Sampling depths were between 0-5 cm; 10-15 cm; 30-35 cm, with a total of 45 average samples being taken, belonging to the following soil types: typical preluviosol (SP 1- Management unit UP 1 Veseuș, Forest district - O.S. Aiud); typical luvisol (SP 2 - Management unit - UP II Micăsasa; SP 7 West, SP 8, Management unit - UP III Târnava); typical brown luvisol (SP 10 - Management unit UP VII Moșna); calcaricregosol (SP 6 - Management unit UP III Târnava, SP 15, 16 Management unit UP I Șeica Mică); stagnant luvisol (SP 7 East- Management unit - UP III Târnava); marnic-phaeozem (SP 9 - Management unit UP III Târnava; SP 12, 13, 14 - Management unit UP I Șeica Mică, Forest district O.S. Mediaș) (Florea and Munteanu, 2003; [xxx], 2008; [xxx], 2018; [xxx], 2020).

The rules for soil samples processing issued by ICPA Bucharest ([xxx], 1981) were observed, with compulsory preparatory stages for the selection and removal of non-decomposed plant remains,

gravel and foreign materials; indoor drying with drying racks and ventilation system was used; grinding the samples was made via a laboratory mill, and sieving on a 2 mm mesh sieve; samples was stored in an hermetically sealed and labelled plastic boxes (by mentioning SP number, and sampling date).
To assess the trophicity of the soil and the bioavailability of some macronutrients for plants in the context of the historical pollution of the Copșa Mică area, the analytical determinations aimed at assessing the content of humus, total N, P, and mobile (extractable) K.

Determination of organic C level and the estimation of humus content was carried out by the Schollenberger-Jackson method (Târziu and Spârchez, 1987) which is a hot wet oxidative method and consists in the digestion of soil samples with an oxidizing mixture $K_2Cr_2O_7$ - H_2SO_4 (Târziu and Spârchez, 1987) the cooled digestate is titrated with a 0.2N Mohr's acid solution in the presence of the phenyl anthranilic acid as indicator. The source of interference for this method consists of the presence of chlorides or oxidizing or reducing agents in the surveyed sample. The results are expressed in percentage in total humus = % organic C x 1.724 (van Bemmelen factor).

Determination of total N was carried out by the wet Kjeldahl digestion method. The three steps of the method comprise digestion to convert N to HNO_3, distillation to retain the NH_3 formed following digestion, and estimation of NH_3 by volumetric analysis. The method related equipment used was the Kjeldahl digestion apparatus and the NH_3 distillation apparatus.

Determination of mobile (accessible) P from soil by the Egner-Riehm-Domingo method consists in the formation of coloured compounds in reaction with specific reactants whose intensity is proportional to the concentration of the determined element. The mobile (accessible) phosphates in the soil were extracted with an ammonium lactate (AL) solution (Mamo et al., 1996) buffered with acetic acid at pH 3.75.

Determination of mobile (accessible) K by the Egner - Riehm - Domingo method is based on the passage of excess H^+ and NH_4^+ from the extractant solution of ammonium acetate-lactate (AL), upon exchange, with K^+ from the exchangeable form of the soil. The dosage of these ions is carried out using the acetylene flame atomic absorption spectrometry (FAAS) (Jeffery et al., 1989).

RESULTS AND DISCUSSIONS

Total humus (%) was determined at depths of 0-5 cm and 10-15 cm. At the former sampling depth, the variation amplitude of the total humus content is between 0.12% (poorly humiferous) and 37 % (excessively humiferous), 31% of the samples having a moderate total humus content, and 23% being weakly humiferous. At a depth of 10-15 cm, the soil is weakly humic in proportion to 31%, (Figure 1 shows a downward trend as the depth increases).

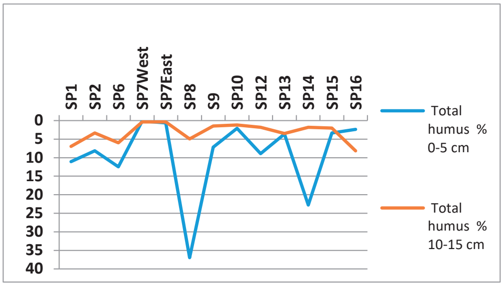

Figure 1. Variation of total humus (%) content between SPs in the experimental sites

The exceptions occurred in SP 16 where a reversal of the downward trend is obvious. The excessively humic soils belong to SP 8 and SP 14 (SP 8 is very polluted, affected by a forest fire produced in a year before the sampling, and SP 14 is located to the SW of Copșa Mică, in the Visa valley, the soil sampling being carried out in area with rich acacia and hawthorn litter). The sample areas in the Târnăvioara Improvement Perimeter, show highly degraded and polluted soils are consequently very poor in humus.
From the analysis of the correlation coefficients of the total humus content with the Cd concentrations established from the same samples, the mathematical connection between the humus content and the Cd content resulted only in the first depth step (Table 1), confirming the protective, sorbent and immobilizing role of humus against toxic substances entering the soil (Ge and Hendershot, 2005).

Table 1. Values of correlation coefficients between the total humus content and the concentrations of Cd and Pb in the sampled soils

Main characteristic	Sam-pling depth	Heavy metal determined	Consis-tency factors *r*	*T test*	Level of significance
Total humus (%)	0-5 cm	Cd	0.89	6.76	***Significant***
	10-15 cm		-0.02	-0.08	ns
	30-35 cm		-0.34	-1.24	ns

Figure 2 shows the values of the total N content determined at the depths of 0-5 and 10-15 cm. The comparison with the content classes according to ICPA (1987) shows that in the heavily polluted areas (SP7East and SP7West) located on the territory of the Ecological Reconstruction Perimeter Târnăvioara, near the main polluter, the total N content of the soil is low (lower than the values presented by Alexa et al. (2004) which are between 0.13-0.27% at the depth of 0-25 cm), and the humus content is reduced due to surface erosion combined with frequent landslides, especially in SP 7West.

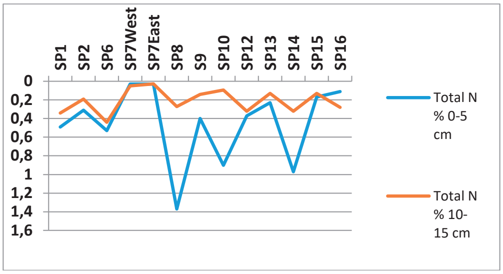

Figure 2. Total N content (%) of the surveyed soils at the first two sampling depths

Average content of total N was determined in SP 15 located at the edge of national road DN 14B, also affected by emissions from road traffic. SP 2, 9, 13, shows a lower concentration of total N. High values were quantified at the 0-5 cm depth of sample plots no. 8, 10, and 14. The soil supply of total N from the control sample plot is high in the case of the two sampling depths (Figure 2). The values of the weighted C/N ratio of the surveyed soil samples do not exceed the value of 17 (Figure 3), which shows a good supply of N. According to Xu et al. (2013), values of C/N ratio <20 indicate a positive mineralization of N. A low C/N ratio indicates the release of significant amounts of soluble N that can be lost from the soil through various ways (Qafoku et al., 2000) which are reflected in the low total N content of SP 7 East and SP 7 West, SP 9, SP 12, SP 14, and SP 15 with C/N ratio <10. In SP 7 East and SP 7 West of the Târnăvioara Improvement Perimeter, mineralized N is lost due to soil erosion and surface soil washing.

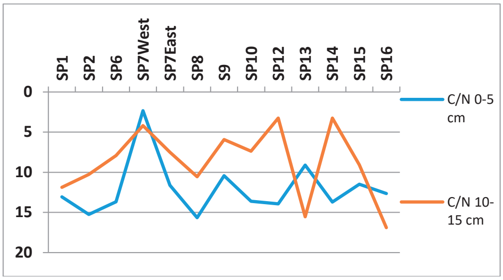

Figure 3. Weighted average values of the C/N ratio at the sampling depth of 0-15 cm in relation to the sample plots

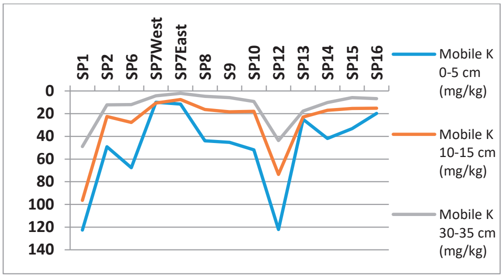

Figure 4. The mobile K content (mg/kg) of the soils surveyed at sampling depths.

Figure 4 highlights maximum values of mobile K at the first depth level, which follows an obvious decreasing trend in terms of depth so that based on the limits of interpretation of the state of supply of soils with mobile K (ICPA, 1981) at the depth of 10-15 cm, the determined values fall into the class of extremely low and low content, and for the depth of 30-35 cm between extremely low and very low content. By comparing the weighted average values at 0-35 cm between the SP, SP 7 East and SP 7 West from the Târnăvioara Improvement Perimeter strongly affected by pollution and erosion with the poorest supply of mobile K stand out clearly, 90% of the SP being marked by an extremely low K content (<40 mg K/kg, ICPA, 1981).
In the soil sampled from the first sampling stage in SP 8 and SP 13, the concentration of mobile P is very high (Figure 5), the remaining values ranging between extremely low and medium content classes (ICPA, 1981). Control SP 1 shows an extremely low concentration of mobile P.

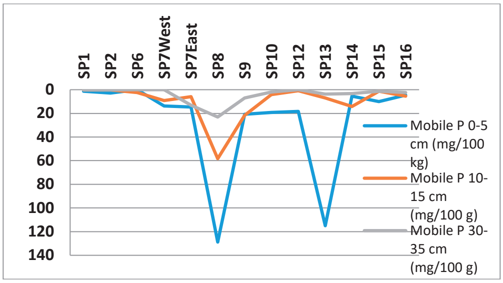

Figure 5. The degree of mobile P supply of the surveyed soils according to SP and sampling depth

The very high concentration established in SP8 may be caused by the input of P originating from the mineralization of the plant material following a forest fire started one year before the collection of the soil samples. For the second and third sampling depths, a general trend of decreasing values is observed in relation to the first depth (except for SP 14 and SP16 and SP7East from the Târnăvioara Improvement Perimeter strongly affected by erosion and horizon inversions (a phenomenon also noted by Smejkal (1982) and Alexa et al.(2004) with very low content specific value)). In the sample plots within the experimental site, soils with extremely low and very low content of average mobile P are dominant (i.e. 66.66%).

CONCLUSIONS

Although the literature on the soils of the Copșa Mică area is substantial, we consider it inappropriate, even inadequate, to compare and report the data of this study with existing values and results due to the inconsistency of the sampling depths. At an overview, there are highlighted both the SP7East and SP7West from the territory of the Târnăvioara Improvement Perimeter located in the vicinity of the main source of pollution within the area with very weak humic soils and a content of very low total N, low mobile P and mobile, bioavailable K, which is very small at all sampling depths, these outcomes being to some extent consistent with the results of the research conducted by Alexa et al. (2003) which characterizes the area as excessively or strongly eroded with sloughs in some places, bare or grassy terrain, and with de-structured and slippery soil.

The aforementioned elements, as a result of the aggressiveness of pollutants on the frequent edaphotopes, especially in SP 7 East and SP 7 West, depletes the superficial soil in humus that contains large amounts of assimilable N, decreasing its trophicity degree. Sample plot 1 (SP 1) considered as a control plot located in an area protected against the influence of a major polluter presents a high total N content, an extremely low mobile K content, and a very low mobile P. Field data and observations show that the presence of rich litter or forest fires can increase the content of N, P or humus in the soil (see cases of SP 8 and SP 14). The content of mobile K is extremely low at a depth of 0-5 cm and extremely low to very low at a depth of 30-35 cm, the downward trend in terms of analytical values while going in depth being obvious. The same downward trend also is present in the case of total N (except in SP 16), mobile P except SP 7 East and SP 14 and total humus, less in SP 7 East; SP 7 West and SP 16. Excessively humic soils are found in sample plots with rich litter or with exogenous C supply following a forest fire.

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THE INFLUENCE OF SOIL TILLAGE SYSTEM AND FERTILIZATION ON THE DEVELOPMENT AND YIELD OF GRAIN SORGHUM IN THE CONDITIONS OF SĂRĂŢENI, IALOMIŢA

Andrei BĂNICĂ, Doru Ioan MARIN

University of Agronomic Sciences and Veterinary Medicine of Bucharest,
59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: andreibanica1995@gmail.com

Abstract

Drought is one of the abiotic stress factors that affect cereal production and represents an important risk factor even for tolerant crops, especially for spring crops. Climate change is putting more and more pressure on the cultivation technology of agricultural land, for which it is necessary to adapt the cultivation technology. This paper presents the results of the research carried out in the period 2020-2022, with the aim of identifying the best interactions between different variants of the basic soil work and some fertilization funds based on nitrogen, phosphorus and foliar fertilizers, thus it will be possible to establish, for the area under study (Sărăţeni-Ialomiţa county), in non-irrigated conditions of sorghum culture, which are the optimal options for obtaining a high production. Analyzing the data from the two years of research, 2021 a year rich in precipitation and 2022 a year with a water deficit, the influence of climatic conditions can be observed. The deeper the loosening of the soil was, the higher the increase in yeald was, tillage by scarification at 35 cm and at 45 cm excelled at all four fertilizations, thus having the highest biomass growth rates in the sorghum crop. The most favorable combination of technological factors that ensured a maximum production yield in 2021 of 8,040 kgha^{-1}, was represented by tillage by scarifying at 45 cm and a fertilization of $N_{100}P_{50}$.

Key words: *Sorghum bicolor L., soil tillage, fertilization, yield.*

INTRODUCTION

According to the purpose for which it is grown, sorghum is divided into four groups: grain sorghum (*Sorghum bicolor* var. *eusorghum*), technical sorghum (for brooms, pulp and paper) (*Sorghum bicolor* var. *technicum*), sugar sorghum (*Sorghum bicolor* var. *saccharatium*) and fodder sorghum (*Sorghum bicolor* var. *sudanense*) (Roman et al., 2006). Therefore, the sorghum culture has multiple and extensive uses on almost all continents, being the staple food of several people (Starodub, 2008).

Sorghum is the main cereal of Africa, due to its high fluoride content, contributing to the enviable appearance of the dentition of the African population (Ștefănescu et al., 2001). In Africa, it is used directly in human nutrition in the form of flour (Axinte et al., 2006).

Sorghum is also cultivated for green fodder or silage, constituting an important source of fodder for animals (Cernea, 2003). Sorghum plants have an increased resistance to drought, heat and make good use of poorly productive soils (Bucur, 2014). The drought tolerance of sorghum is attributed to a dense and deep root system capable of extracting water from deep in the soil (Wright and Smith, 1983; Singh and Singh, 1995), its ability to keep stomata open at low water potential by osmotic adjustment (Ludlow and Muchow, 1990), its ability to produce in a wide range of soil types, thus advancing different growth situations (Lafarge and Hammer, 2002) and the ability to delay reproductive development (Wright et al., 1983).

In contrast, maize is significantly affected by water deficit and other environmental factors around anthesis (NeSmith and Ritchie, 1992a; NeSmith and Ritchie, 1992b; Otegui et al., 1995; Andrade et al., 1999). A recent study suggested that similar transpiration but different transpiration rates influence their comparative adaptation to rainfall-limited environments (van Oosterom et al., 2020).

Research on the influence of culture technology on sorghum production, carried out in different areas, shows that it reacts favorably to mineral fertilization (Oprea et al., 2017).

In this context, the objective of the research carried out and provided in the paper is to take into account the main technological elements that influence a high production in the culture of sorghum for grains: the basic work of the soil and fertilization with a view to an optimal level of yield in correlation with the conditions in Sărăţeni locality, Ialomiţa county.

MATERIALS AND METHODS

The researches were carried out in the town of Sărăţeni-Ialomiţa county (44°38′11″N 26°55′41″E) between the years 2020-2022 within the Bănică Ion Individual Enterprise on a cambic chernozem. The rainfall regime was high in 2021, with a total of 673.6 mm, thus favoring obtaining a high production. Both at sowing and during the important phases of development, there was an amount of precipitation above normal. During the period of vegetative development of plants (May-August), 280 mm were recorded in 2021, and 96 mm in 2022, the difference between the two years being 184 mm (Table 1). The average monthly temperature for the period of vegetative growth is 20.8°C, and the average for the period 2020-2022 is 22.7°C, which shows us that the area is consistent with global warming.

Table 1. Climatic conditions during sorghum plant's vegetative period at Sărăţeni-Ialomiţa

	Temperature (ºC)			Rainfall (mm)		
	Normal (1981-2010)	2020-2021	2021-2022	Normal (1981-2010)	2020-2021	2021-2022
Octomber	11.4	14.4	9.8	37.9	24.4	58.6
November	5.0	5.6	7.2	34.6	15.9	37.5
December	0.0	3.9	2.3	33.4	84.1	56.2
January	-1.1	1.9	1.9	23.6	78.7	3.8
February	0.4	2.8	4.2	22.3	23.5	3.7
March	5.3	4.9	4.0	27.2	77.0	13.0
April	11.4	9.5	11.8	41.1	51.0	38.0
May	17.6	17.0	17.8	50.5	36.0	15.0
Jun	21.4	20.7	22.7	71.2	175.0	26.0
July	23.2	24.9	25.5	61.9	33.0	31.0
August	22.6	23.6	24.8	46.8	36.0	24.0
September	17.3	16.8	17.2	47.8	3.4	0.0
Avg. (ºC) Sum (mm)	11.2	12.1	12.4	498.3	638.0	306.5

The experiment was placed in randomized blocks, in 3 repetitions with the analyzed factors: Factor A-basic soil work with graduations: a_1-Plow at 25 cm; a_2-Scarified at 35 cm; a_3- Scarified at 45 cm; a_4-Disc 10 cm; and factor B - fertilizations with graduations: b_1- N_0P_0 (Control); b_2- $N_{100}P_0$; b_3-$N_{100}P_{50}$; b_4-Borocal 1.5 l/ha (Foliar).

The biological material studied was the hybrid ES Abanus, a simple hybrid with white grains, excellent vigor when starting vegetation with compact panicles and very good tolerance to drought and shaking. It shows high tolerance to *Fusarium*, the plant size is small with very good resistance to falling.

Tillage and fertilization were applied according to the graduations. The predecessor plant was corn.

Chemical fertilizers were administered before sowing, and foliar fertilization was applied to the vegetation.

Sowing was carried out at a density of 230,000 grains/ha, and after sowing pre-emergent herbicide was used with Dual Gold 960 EC herbicide at a dose of 1.2 l/ha.

RESULTS AND DISCUSSIONS

The influence of fertilization on the height of sorghum

The influence of fertilization on the height of sorghum, carried out in June, includes the period of intense vegetative growth in all experimental variants. The plants had a height between 70.8 cm in the non-fertilized-plowed version and 95.5 cm, in the version fertilized with foliar fertilizers-scarified at 45 cm (Table

2). Compared to the unfertilized control, the application of fertilizers determined the obtaining of more vigorous plants, the best results were found in the case of the variants fertilized with nitrogen and phosphorus, tillage by scarification at 35 cm and at 45 cm.

Fertilizing the soil with phosphorus-based fertilizers brought the biggest differences compared to the non-fertilized variants, the largest being 9.1 cm for the variant a_1- Plow 25 cm + b_3- $N_{100}P_{50}$.

By comparison with the unfertilized control variant, against the background of the application of nitrogen ($N_{100}P_0$) and phosphorus ($N_{100}P_{50}$) fertilization, the plantsa had lower heights, but a higher twinning.

Table 2. The influence of the agrofund on the height of sorghum plants in different tillage systems

Variant	Year	b1 - N_0P_0			b2 - $N_{100}P_0$			b3 - $N_{100}P_{50}$			b4 - Foliar (Borocal 1.5 l/ha)		
		cm/plant	%	Dif.	cm/plant	%	Dif.	cm/plant	%	Dif.	cm/plant	%	Dif.
a1 - Plow 25 cm	2020-2021	70.8	100	C	79.5	112.2	8.7	79.9	112.8	9.1	78	110.1	7.2
	2021-2022	83.4	100	C	86.1	103.2	2.7	85.9	102.9	2.5	85.2	102.1	1.8
	Avg.	77.1	100	C	82.8	107.3	5.7	82.9	107.5	5.8	82.8	107.3	5.7
a2 - Scarified 35 cm	2020-2021	80.4	100	C	76.3	94.9	-4.1	76.1	94.6	-4.3	80.5	100.1	0.1
	2021-2022	83.3	100	C	84.5	101.4	1.2	87.6	105.1	4.3	85.1	102.1	1.8
	Avg.	81.8	100	C	80.4	98.2	-1.4	81.8	100	0	82.8	101.2	1
a3 - Scarified 45 cm	2020-2021	80.6	100	C	85.2	105.7	4.6	76	94.2	-4.6	88.2	101.9	7.6
	2021-2022	92.4	100	C	89	96.3	3.9	92.5	100.1	0.1	95.5	103.3	3.1
	Avg.	86.5	100	C	87.1	100.6	0.6	84.2	97.3	-2.3	91.8	106.1	5.3
a4 - Disc 10 cm	2020-2021	78.9	100	C	73.9	93.6	-5	72.3	91.6	-6.6	88.2	103.1	9.3
	2021-2022	81.5	100	C	80.5	98.7	-1	83.9	102.9	2.4	81.4	99.8	-0.1
	Avg.	76.2	100	C	77.2	101.3	1	78.1	102.4	1.9	81.4	106.8	5.2

Deeper tillage of the soil increases the height of the sorghum plants, regardless of the way it was fertilized (Table 3). The highest values were recorded with scarified tillage at 45 cm: 113.8 cm, unfertilized, compared to the variant worked by discus: 78.9 cm. Scarification at 35 cm and at 45 cm brought similar values regarding the height of sorghum plants to all three fertilization methods.

The deep tillage of the soil by scarification determined a better utilization of the foliar fertilization in the two experimental years, the plants had heights of 10.2 cm and 10.3 cm compared to the conventional tillage system.

Tabel 3. The influence of tillage on the height of sorghum plants through different types of fertilization

Variant	Year	b1- N_0P_0			b2- $N_{100}P_0$			b3- $N_{100}P_{50}$			b4- Foliar(Borocal 1.5 l/ha)		
		cm/plant	%	Dif.	cm/plant	%	Dif.	cm/plant	%	Dif.	cm/plant	%	Dif.
a1 - Plow 25 cm	2020-2021	70.8	100	Ct	79.5	100	Ct	79.9	100	Ct	78	100	Ct
	2021-2022	83.4	100	Ct	86.1	100	Ct	85.9	100	Ct	85.2	100	Ct
	Avg.	77.1	100	Ct	88.2	100	Ct	82.9	100	Ct	82.8	100	Ct
a2 - Scarified 35 cm	2020-2021	80.4	113.5	9.6	76.3	95.9	-3.2	76.1	96.3	-3.8	80.5	103.1	2.5
	2021-2022	83.3	99.8	-0.1	84.5	98.1	-1.6	87.6	101.9	1.7	85.1	99.8	-0.1
	Avg.	81.8	106.1	4.7	80.4	91.1	-7.8	81.8	98.6	-1.1	82.8	100	0
a3 - Scarified 45 cm	2020-2021	80.6	113.8	9.8	85.2	107.1	5.7	76	95.1	-3.9	88.2	113	10.2
	2021-2022	92.4	110.7	9	89	103.3	2.9	92.5	107.6	6.6	95.5	112	10.3
	Avg.	86.5	112.2	9.4	87.1	98.7	-1.1	84.2	101.5	1.3	91.8	110.8	9
a4 - Disc 10 cm	2020-2021	78.9	111.4	8.1	73.9	92.9	-5.6	72.3	90.4	-7.6	88.2	113	10.2
	2021-2022	81.5	97.7	-1.9	80.5	93.4	-5.6	83.9	97.6	-2	81.4	95.5	-3.8
	Avg.	80.2	98.8	-0.9	77.2	87.5	-11	78.1	94.2	-4.8	81.4	98.3	-1.4

The influence of fertilization on sorghum production

Based on the application of 100 kg of nitrogen/ha, an average increase in production of 2,779 kg was recorded compared to the unfertilized control in the variant of tillage by scarifying at 45 cm and an increase of 1,987 kg/ha when scarifying the soil at 35 cm. Foliar fertilization of sorghum brought the smallest increase in production, the maximum difference compared to the control being 674 kg in the scarified version at 35 cm. Analyzing the averages over the two years, the b2-$N_{100}P_0$ variant brought increases starting with 1,987 kg when tilling the soil by discus, up to a maximum increase of 2,779 kg when tilling the soil by scarifying at 45 cm. The application of nitrogen and phosphorus in the sorghum culture greatly influenced the production, the interaction between $N_{100}P_{50}$ and scarified at 45 cm brought the highest level of harvest of 5,525 kg, with an increase in production compared to the unfertilized control: 3,164 kg (Table 4). Analyzing tillage, production increased gradually with deeper tillage. The highest production, on average for all experimental variants, was recorded in the work by scarification at 45 cm of the land together with fertilization with $N_{100}P_{50}$: 8,040 kg/ha.

Deep loosening of the soil gradually increased the production of grain sorghum. Scarification of the soil at 45 cm proved to be the most productive choice even in a year with low precipitation (2022-306.5 mm), where a minimum production of 1,915 kg and a maximum of 3,010 kg was obtained (Table 5). In terms of tillage, production increases start from 102 kg (scarified at 35 cm + foliar fertilization) and reach an average maximum of 884 kg (scarified at 45 cm +$N_{100}P_0$).

Table 4. The influence of the agrofund on the height of the plants

Variant	Year	b1 - N_0P_0			b2 - $N_{100}P_0$			b3 - $N_{100}P_{50}$			b4 - Foliar (Borocal 1.5 l/ha)		
		kg/ha	%	Dif.	kg/ha	%	Dif.	kg/ha	%	Dif.	kg/ha	%	Dif.
a1 - Plow 25 cm	2020-2021	2.986	100	Ct	6.753	226.1	3.767	6.900	231	3.914	3.316	111	330
	2021-2022	1.176	100	Ct	1.760	149.6	584	2.500	122.5	1.324	1.331	113.1	155
	Avg.	2.081	100	Ct	4.256	204.5	2.175	4.700	225.8	2.619	2.323	111.6	242
a2 - Scarified 35 cm	2020-2021	2.706	100	Ct	7.490	276.7	4.784	7.420	274.2	4.714	3.380	124.9	674
	2021-2022	1.550	100	Ct	2.103	35.6	553	2.480	160	930	1.610	103.8	60
	Avg.	2.128	100	Ct	4.796	225.3	2.668	4.950	232.6	2.822	2.425	113.9	297
a3 - Scarified 45 cm	2020-2021	2.808	100	Ct	7.715	274.7	4.907	8.040	286.3	5.232	3.402	121.1	594
	2021-2022	1.915	100	Ct	2.565	133.9	650	3.010	157.1	1.095	2.185	114	270
	Avg.	2.361	100	Ct	5.140	217.7	2.779	5.525	234	3.164	2.793	118.2	432
a4 - Disc 10 cm	2020-2021	2.600	100	Ct	6.100	234.6	3.500	6.640	255.3	4.040	2.700	103.8	100
	2021-2022	956	100	Ct	1.430	149.5	474	1.673	175	717	1.116	169	160
	Avg.	1.778	100	Ct	3.765	319.6	1.987	4.156	352.8	2.378	1.908	161.9	130

Table 5. The influence of tillage and fertilization on yield in 2021-2022

Variant	Year	b1 - N_0P_0			b2 - $N_{100}P_0$			b3 - $N_{100}P_{50}$			b4 - Foliar (Borocal 1.5 l/ha)		
		kg/ha	%	Dif.	kg/ha	%	Dif.	kg/ha	%	Dif.	kg/ha	%	Dif.
a1 - Plow 25 cm	2020-2021	2.986	100	Ct	6.753	100	Ct	6.900	100	Ct	3.316	100	Ct
	2021-2022	1.176	100	Ct	1.760	100	Ct	2.500	100	Ct	1.331	100	Ct
	Avg.	2.081	100	Ct	4.256	100	Ct	4.700	100	Ct	2.323	100	Ct
a2 - Scarified 35 cm	2020-2021	2.706	90.6	-280	7.490	111	737	7.420	107.5	520	3.380	110	64
	2021-2022	1.550	131.8	374	2.103	119.4	343	2.480	99.2	-20	1.610	121	279
	Avg.	2.128	102.2	47	4.796	112.6	540	4.950	105.3	250	2.425	104.3	102
a3 - Scarified 45 cm	2020-2021	2.808	94	-178	7.715	114.2	962	8.040	116.5	1140	3.402	102.5	86
	2021-2022	1.915	162.8	739	2.565	145.7	805	3.010	120.4	510	2.185	164.1	854
	Avg.	2.361	113.4	280	5.140	120.7	884	5.525	117.5	825	2.793	120.2	470
a4 - Disc 10 cm	2020-2021	2.600	87	-386	6.100	90.3	-653	6.640	96.2	-260	2.700	81.4	-616
	2021-2022	956	81.2	-220	1.430	81.2	-330	1.673	67	-827	1.116	83.8	-215
	Avg.	1.778	56.6	-303	3.765	88.2	-791	4.156	88.4	-544	1.908	82.1	-415

CONCLUSIONS

Following the research carried out, the following more important conclusions can be summarized regarding the influence of tillage systems and fertilization in grain sorghum culture:

Under the conditions of a cambic chernoziom in the Sărăţeni-Ialomiţa area, the highest height of the sorghum plants was obtained in the variants fertilized with foliar fertilizers, together with tillage by scarification.

With the deeper work of the soil, the height of the sorghum plants also increases, regardless of how it was fertilized.

The biggest increase in production of 3,164 kg was recorded applying a fertilization with $N_{100}P_{50}$. Fertilization only with nitrogen brought an average increase in the sorghum culture for grains of 2,779 kg.

The deep tillage of the soil by scarification at 45 cm brought the highest production on average over the two years: 5,525 kg, with an increase of 884 kg/ha, compared to the control plowed at 25 cm.

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THE INFLUENCE OF STORAGE CONDITION ON MYCOTOXIN LOAD ON MAIN FEED INPUTS IN SOUTHEAST ROMANIA

Silviu-Ionuţ BEIA, Elena Ştefania IVAN, Costel MIHALAŞCU, Roxana CICEOI

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: elena.ivan@qlab.usamv.ro

Abstract

Mycotoxins are toxic compounds for animals and humans, naturally produced by different types of fungi. Exposure to mycotoxins through the consumption of contaminated food or feed leads to gastrointestinal and renal disorders to the point of immune deficiency and cancer. Most mycotoxins are chemically stable and persist in food processing. Fusarium graminearum is a dangerous pathogen of the cereals producing mycotoxins (trichothecene and zearalenone) harmful for human and animal health. Given the implementation of the requirements of the European Green Deal, especially those related to organic products, and in the context of climate change, especially the fluctuations in temperature and humidity, the increase of the presence of mycotoxins in food and feed is expected. In these conditions, the evaluation of the degree of mycotoxin contamination in different steps on the food chain, with fast and accurate methods, is very important. The aim of this work was to investigate the dynamics of fungi and related mycotoxins on wheat, maize and sunflower during cereal storage in vertical and classic silos.

***Key words**: Ochratoxin A, Fusarium graminearum, wheat, storage.*

INTRODUCTION

Cereals play a critical and irreplaceable role in both agricultural production and nourishing the global population (Tremmel-Bede et al., 2022). In developed nations where cereal grain production is significant for both food and feed purposes, substantial quantities of grains are retained in storage for extended periods, often exceeding a year, to meet the demands of domestic cereal industries and fulfill import and export requirements. As defined by Dunkel (1992), the concept of stored grain ecosystems revolves around dormant autotrophs, which are the seeds themselves. These seeds serve a dual purpose, functioning as both an energy source and a habitat for a diverse array of heterotrophic organisms, including fungi, bacteria, insects, and mites (Fleurat-Lessard, 2017).

In adverse field conditions that pose stress to crops, certain fungi have the capability to produce mycotoxins. These mycotoxins are secondary metabolites that have the potential to taint a variety of agricultural products, both in the field and during storage (Yao et al., 2015). Among the crops commonly vulnerable to contamination by aflatoxins, we find cereals such as maize, rice, and wheat (Shephard et al., 2013). Additionally, it's worth noting that there are fungi that infect seeds and serve as sources of specific mycotoxins (Chulze, 2010; Ittu, 2006). These mycotoxins can pose significant risks to consumers within the food chain.

The interconnection between well-being in individuals, the well-being of societies, and the well-being of the planet underscores the significance of sustainable food systems within the framework of the European Green Deal, the European Union's strategy for sustainable and equitable development. This strategy is crafted to invigorate the economy, enhance the health and well-being of individuals, and uphold the preservation of nature. The European agricultural and food system, underpinned by the Common Agricultural Policy, already serves as a global benchmark for its commitment to safety, supply reliability, nutritional value, and overall quality (CAP strategic plans, European Commission, europa.eu, 2023). Mycotoxins are toxic compounds generated by specific fungal species that thrive on grain and feed products when stored under unfavorable moisture conditions. These mycotoxins possess the potential to yield severe consequences for both human and animal well-being. To mitigate the risk of introducing mycotoxin-contaminated food and feed into the grain processing chains, the European Union has implemented several regulations (European Commission, 2006, 2007, 2010; Meucci et al., 2021; Codex Alimentarius Commission, 2014). The practice of storing cereals dates back to ancient times, primarily due to the seasonal nature of cereal crops that are consumed year-round. For strategic purposes and to minimize transportation needs, it is advisable to decentralize and distribute grain storage across a broad geographic area, utilizing the grain close to its point of production (De Martini et al., 2009). Typically, cereal storage takes place in round (or occasionally square) bins or silos constructed from materials such as metal or cement. These storage facilities come in various capacities and are equipped with sophisticated infrastructure (Silva, 2002; Silva et al., 2012). Stored maize represents a constructed ecosystem in which alterations in quality and nutritional content take place due to the intricate interplay among physical, chemical, and biological factors. A significant concern within this context revolves around fungal spoilage and the potential contamination of mycotoxins. Fungal infections can lead to mycotoxin contamination at various stages, including growth, harvest, storage, transportation, and processing (Chulze, 2010).

Metal silos, characterized by their cylindrical shape and hermetic sealing, are constructed using galvanized iron sheets. This technology has proven highly effective in safeguarding harvested grains against infestations by storage insects and rodent pests. The hermetic nature of metal silos ensures the absence of oxygen inside, which leads to the demise of any internal insect pests. Furthermore, it serves as a complete barrier against any pests or pathogens that might attempt to infiltrate the grain (Terefa et al., 2011). To address concerns related to animal and human health, maximum allowable limits for *Fusarium* mycotoxins in wheat, maize grain, and their by-products have been established globally (Machado et al., 2021).

The primary objective of this research endeavor was to conduct an in-depth examination into the ever-changing dynamics of fungal populations and their associated mycotoxins within the context of wheat, maize, and sunflower storage. Specifically, we sought to gain a comprehensive understanding of how these intricate processes unfold when these cereals are stored within both vertical and conventional silos.

MATERIALS AND METHODS

The biological material in this study consisted of five samples each of corn, wheat, barley, and sunflower seeds (Figure 1). These samples were collected from two distinct deposition sites within Ilfov County, specifically from classic silos.

Figure 1. Samples of sun flowers, barley and corn

To prepare the seeds for placement on the culture medium within Petri plates, a series of meticulous steps were followed. First, the grains underwent a thorough washing process with tap water. Subsequently, they were subjected to disinfection by immersion in 96% ethyl alcohol for a duration of one minute, followed by two consecutive washes with sterile water to remove any remnants of disinfectant. The disinfected grains were then carefully dried on sterile filter paper. Once the grains were placed on the culture medium within the Petri dishes, these dishes were incubated at a controlled temperature of 22°C for a period of nine days. Following the incubation period, a comprehensive analysis of the samples was conducted, both through visual inspection and using a stereomicroscope. To facilitate the proper identification of the fungi that had developed on the surfaces of the grains, microscope slides were prepared.

The data we acquired were subjected to an analysis that involved the computation of the fungal contamination index. This index, denoted as ICS%, was calculated utilizing the following formula: ICS% = (number of contaminated seeds/total number of seeds on the plates) x 100. Furthermore, the evaluation of mycelial growth on the grains was conducted under the scrutiny of a stereomicroscope, and this examination process was further complemented by a microscopic assessment. To determine the prevalence of grains affected by *Fusarium*, a comprehensive analysis was performed by considering the proportion of grains exhibiting disease symptoms in relation to the total number of corn grains subjected to analysis.

RESULTS AND DISCUSSIONS

It is firmly established within the scientific community that mycotoxin contamination can manifest in agricultural products both during their growth in the field and subsequent storage.

These analyses were conducted at the diagnostic laboratory dedicated to plant protection, within the Research Center for Studies of Food Quality and Agricultural Products.

To examine the microflora, present in the samples, we employed seeds from wheat, maize, and sunflower, which were placed on PDA culture media. The progression of fungal growth was observed over a span of nine days during the incubation period.

The year 2022 presented significant challenges for the maize crop, marked by instances of heat and water stress during critical phases such as pollination and ripening. The thermal and water conditions recorded during the months of July and August 2022 exerted a detrimental impact on corn development.

As a consequence, the corn harvest in the year 2022 experienced a significant reduction in yields. Regrettably, the limited harvests obtained were also discovered to be tainted with mycotoxigenic fungi. Several critical factors play a role in the development of storage fungi. These factors include the moisture content of the stored grain, the temperature maintained within the grain storage facility, and the duration for which the grain is kept in storage. Naturally, the longer the grain is stored, the higher the susceptibility it bears to fungal infestations during storage. However, it is important to note that the progression of this process is heavily influenced by the specific conditions of storage.

In summary, the contamination of grains by fungal pathogens can occur at multiple stages in the grain's journey from the field to storage. It can initiate during harvest due to improper drying or exposure to moisture, continue during transportation in less-than-ideal conditions, and persist in storage if the environment is conducive to fungal growth. It is crucial to implement proper agricultural and storage practices to minimize the risk of fungal contamination and mycotoxin production in grains, ensuring the safety and quality of the final products for consumption. These developments subsequently lead to grain spoilage. The emergence of storage fungi on seeds results in seed rot, a decline in quality and nutritional value, and the production of mycotoxins by certain fungi, which pose toxic risks to both humans and animals. Grains affected by storage fungi can exhibit discoloration and, in some cases, emit a musty odor. Various fungi have the potential to induce grain spoilage during storage, with the most prevalent species being *Penicillium* spp., *Aspergillus* spp., *Alternaria* spp., *Fusarium* spp., and *Rhizopus stolonifera*. These pathogens naturally occur in the environment but can proliferate in seed lots due to factors such as contaminated equipment or storage bins, mechanical damage to the seeds, and insect infestations.

Table 1 provides data illustrating the microflora associated with wheat, as well as seeds from wheat, maize, barley, and sunflower.

In the process of examining the various pathogens that impact the seeds within our collected samples, we made several key observations. Specifically, we identified a diverse microflora present within these seeds, which included fungal species belonging to the following genera: *Alternaria* spp., *Penicillium* spp., *Rhizopus* spp., and *Fusarium* spp. These fungi have the potential to harm the quality and viability of seeds, and some may also produce mycotoxins that can pose health risks if seeds are used for food or animal feed. Identifying and understanding the presence of these pathogens is essential for implementing effective management strategies to protect seed quality and agricultural productivity.

Table 1. Microflora detected on the studied seeds

Hybrids	The pathogens				
	Alternaria spp	*Penicillium* spp	*Rhizopus* spp	*Fusarium* spp	*Aspergillius* spp
Glossa	-	-	+	+	+
P9889	-	+	+	+	+
Experto	+	-	+	-	-
Carioca	+	+	+	-	+
Cardinal	-	+	+	+	+

The examination of seeds harvested in the year 2022 revealed a presence of various micromycetes in the studied hybrids. These micromycetes included *Alternaria* spp., *Penicillium* spp., *Rhizopus* spp., and *Fusarium* spp. Within this spectrum, *Penicillium* spp. and *Rhizopus* spp. were specifically identified on sunflower achenes, while the micromycete *Alternaria* spp. was observed on the caryopsis of wheat grains. Additionally, fruiting bodies associated with the genus *Fusarium* spp. were identified on the corn grains. This comprehensive analysis underscores the diversity of micromycetes present across different seed types within the 2022 harvest.

The presence of fusarium wilt in the 2022 corn crop is believed to be primarily attributed to climate changes, specifically characterized by elevated temperatures and prolonged periods of drought. This combination of factors can significantly contribute to contamination by *Fusarium* and *Penicillium*. Notably, the critical period for such contamination is during the ripening phase of corn. The summer of 2022 witnessed a particularly dry climate, marked by exceptionally high maximum temperatures, which likely exacerbated these conditions. In light of these environmental factors, it becomes increasingly essential to assess the extent of mycotoxin contamination through methods that are both rapid and precise. The phenomenon of global warming intensifies the susceptibility to fungal contamination, particularly in regions with warmer climates. This heightened temperature environment increases the risk of aflatoxin contamination in crops like corn and wheat. Aflatoxins are potent toxins produced by certain molds, such as Aspergillus, and they can proliferate more rapidly and abundantly in higher temperatures. The consequences of aflatoxin contamination are significant. It can render these crops unsuitable for use in both human food and animal feed. When crops are tainted with aflatoxins, they become a health hazard, as consuming contaminated food products can lead to severe health issues, including liver damage and an increased risk of cancer. Additionally, contaminated crops cannot be safely used as animal feed, as they can harm livestock and even enter the food chain indirectly through animal products like milk or meat. In summary, global warming exacerbates the risk of aflatoxin contamination in crops due to the elevated temperatures it brings, posing a serious threat to food safety and agricultural sustainability. This issue necessitates close monitoring and mitigation efforts to ensure the security of our food supply (as illustrated in Figure 2). This underscores the urgency of addressing the impact of climate change on crop health and safety.

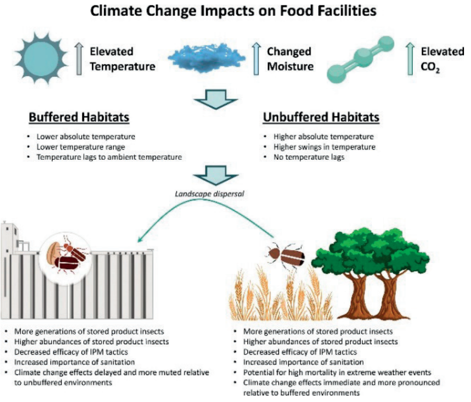

Figure 2. Climate change impact on food facilities (source: Gerken & Morrison, 2022)

The emergence of mycotoxins produced by *Fusarium* head blight (FHB) in wheat grains can be attributed to a multifaceted interplay of various factors. Among these factors, the climatic context plays a pivotal role, encompassing elements such as temperature, humidity, and precipitation patterns. Additionally, the choice of wheat variety, the specific cropping techniques employed, and the precision and reliability of mycotoxin measurement methodologies all contribute significantly to the complex matrix influencing mycotoxin occurrence in wheat grains. In summary, the occurrence of mycotoxins in wheat grains is influenced by a complex interplay of factors, including the choice of wheat variety, agricultural practices, and the accuracy of mycotoxin measurement methods. Furthermore, *Aspergillus* and *Fusarium* species, known pathogens in wheat, corn, and barley, can produce mycotoxins that pose serious health and safety risks, emphasizing the importance of monitoring and management in agriculture.

The most common isolated pathogenic species belong to the genus *Aspergillius* spp and *Fusarium* spp and have colonized the wheat seeds, corn and barley (Figure 3). The *Alternaria* spp fungus was identified on the wheat seeds and sun flowers. The *Peniciullium* spp fungus was identified on the Carioca and Cardinal strains, and the *Fusarium* spp fungus was identified on the Glosa variety (Figure 4). The *Rhizopus* spp fungus were identified on the Glossa, Experto and Cardinal.

Figure 3. *Aspergillus* spp. isolates from P9889

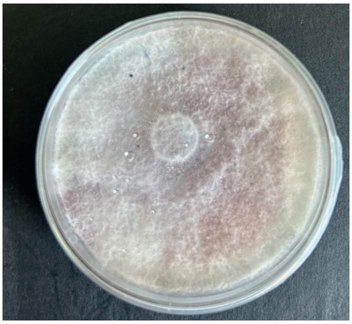

Figure 4. *Fusarium* spp. isolates from Glossa

CONCLUSIONS

In conclusion, our research underscores the dynamics of mycotoxin contamination in agricultural products, which can originate both in the field during growth and during storage. The year 2022 posed significant challenges for corn cultivation due to the adverse effects of high temperatures and prolonged dry periods, particularly during critical phases like pollination and ripening, which had an impact also on contaminatin with mycotoxigenic fungi. Prevalent species responsible for grain spoilage were *Penicillium* spp., *Aspergillus* spp., *Alternaria* spp., *Fusarium* spp., and *Rhizopus stolonifera*. Warmer climates pose increased risks of aflatoxin contamination in crops like corn and wheat, potentially rendering them unfit for consumption or use as animal feed. Thus, addressing the impact of climate change on crop health and safety becomes an imperative.

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PRODUCTION AND SUGAR CONTENT OF FOUR SUGAR BEET HYBRIDS FUNCTION OF ENVIRONMENTAL TEMPERATURE, PRECIPITATIONS AND AGRICULTURAL KEY INPUTS

Letiția Adriana BOCOȘ[1], Antonia ODAGIU[1], Camelia OROIAN[2], Cristian IEDERAN[1], Petru BURDUHOS[1]

[1]University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Faculty of Agriculture, 3-5 Calea Manastur, Cluj-Napoca, Romania
[2]University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Faculty of Horticulture and Business in Rural Development,
3-5 Calea Manastur, Cluj-Napoca, Romania

Corresponding author email: antonia.odagiu@usamvcluj.ro

Abstract

Sugar beet yields are highly influenced by a series of factors, both technological and environmental. This study aims to emphasize the effect of the environmental temperature and agricultural key inputs (fertilization and water) on production and sugar content function of sugar beet hybrid in the same growing area. A three factorial experiment was conducted (genotype x irrigation x fertilization) was organized from 2021 to 2022 in the experimental field located in Viișoara commune, Cluj County. Daily temperature and precipitations were monitored. The results of 2-years studies concerning the sugar content and yields for each experimental sugar beet variety (Vanghelis, Tesla, Penalty, and Gorilla) are recorded. The results of our study show that in Vanghelis variety are reported the highest average yields (66.60 t/ha), and sugar content (10.84%). The fresh and dry root yields and sucrose content did not differ significantly among Vanghelis and Gorilla varieties, but significant varieties are reported among these varieties, and the other two, Tesla, and Penalty, respectively. Strong and moderate correlations are identified between sugar beet yields and sucrose content on one hand, and environmental inputs on the other side, for each studied variety.

Key words: *differences, dry matter, fertilizer, irrigation, water.*

INTRODUCTION

The natural stress produced mainly by temperatures and lack of precipitations influences the plants development, including sugar beet, in negative manner at great extent (Verma, and Deepti, 2016). It is well known that water deficiency may cause disorders in plants physiology, morphology, atomic, or even at biochemical cellular levels (Du et al., 2020). High environmental temperatures together with low rainfall regimen also have unfavorably impacts on photosynthesis and protein action in plants. The diminished photosynthesis rate and lack of accessible water in plants, led do the decrease of the rhythm of dry matter production (Gholami and Zahedi, 2019). It is obvious that above mentioned facts could be considered as serious threat for food supplying chain (Okorie et al., 2019; Verma and Deepti, 2016).

Beta vulgaris L. (sugar beet) is a valuable industrial plant, one of the main industrial sugar sources. According to Kühnel et al. (2011), sugar beet has a dry mass content framing within the interval 18%-23%, but much research has been conducted to obtain hybrids with enhanced values. It has variable content in protein (11.50-20.25%), and about 20-21% rough fiber (Berłowska et al., 2016; Ahmed et al., 2020). Besides its use at large scale as rough material for sugar industry, sugar beet is also used as feedstuff for dairy cattle (Münnich et al., 2017). Because of high importance as cash crop, due to its considerable potential for carbohydrate capacity saves, research has been orientated towards identifying new methodologies for improving both plant yields, resistance against mites and pathogens, and also adverse climatic conditions (Mukherjee and Gantait, 2023). In this way, there have been obtained both by selection and genetic engineering hybrids with improved yield potential and resistance against adverse environmental conditions.

The study was conducted to quantify the production performances of Gorilla, Vanghelis, Tesla, and Penalty sugar beet hybrids in terms of dry matter, yield, and sugar yield, and identify the interactions between the above-mentioned traits with environmental temperature and precipitations.

MATERIALS AND METHODS

A three factorial experiment was conducted (genotype x irrigation x fertilization) in two successive years, 2021 and 2022, respectively, in an experimental field located in Viișoara Commune, Cluj County.

The factor genotype has four graduations, meaning the sugar beet genotypes: Gorilla, Vanghelis, Tesla, and Penalty.

Irrigation, the second factor, has two graduations, no irrigation, and irrigation with a watering norm of 600 m^3/ha by round, using 7 rounds by entire vegetation period. The third factor, fertilization has three graduations: no fertilization, fertilization with NPK in ratio of 60-40-40 kg/ha, and NPK in ratio of 180-120-120 kg/ha. The experimental variants are organized as showed in Table 1.

Table 1. The experimental variants

Experimental variant	Description
a_1b_1	No irrigation (a_1)-no fertilization (b_1)
a_1b_2	No irrigation (a_1)-NPK 60-40-40 kg/ha (b_2)
a_1b_3	No irrigation (a_1)- NPK 180-120-120 kg/ha (b_3)
a_2b_1	Irrigation (a_2)- no fertilization (b_1)
a_2b_2	Irrigation (a_2)- NPK 60-40-40 kg/ha (b_2)
a_2b_3	Irrigation (a_2)- NPK 180-120-120 kg/ha (b_3)

The sugar beet was planted at density of 120,000 plants/ha, and soil moisture of 60%. 24 plots sized of 20 m^2, in three replications by each experimental variant were organized for the filed study.

The environmental temperature and precipitations data were obtained from the meteorological station iMETOS 3.3 placed in the experimental field, which perform daily recordings.

Data regarding sugar beet root weight, beet yield, and sugar yield were collected. The sugar beet yield was calculated by dividing the harvested roots weight to the plot area. The sugar was quantified using the methodology described by Legendre et al. (1972), and sugar yield by multiplying the root yield with sugar content.

Because no significant differences were reported between studied parameters were recorded, data concerning the environmental parameters temperature, and precipitations, and experimental factors are expressed as means by experimental years, 2021 and 2022.

The sugar beet root weight, beet yield, and sugar yield were subjected to statistical analysis. Basic statistics was used for the calculations of means, standard error of mean, ad variability. ANOVA analysis of variance was used for emphasizing the differences in genotypes and treatments (different irrigation and fertilization regimens) at 5% probability level. After testing linearity of parameters, the nonparametric Spearman test was used for calculation of correlation coefficient as alternative to Pearson parametric test (Merce and Merce, 2002).

Based on correlation matrix of the environmental factors temperature, and water supply, respectively, on one hand and sugar beet yield, and sugar yield by all hybrids, on the other hand, to know the importance of Sugar was determined according to each studied parameter indicated by its relative loading the eigenvector, Principal Components Analysis was (PCA) performed.

RESULTS AND DISCUSSIONS

During the vegetation period (April - August), the experimental site is characterized by mean temperatures that framed between 8.77°C (April) - 22.6°C (July), and an average of 17,82°C, by entire period (Figure 1), which are optimal for the development of the culture (Guș et al., 2004; Muntean et al., 2001; Pastor, 2002). In both experimental years a scarcity of rainfall water supply is reported and emphasized by a sum of 221.30 mm/year (Figure 2), which according to sugar beet production technology is very poor, irrigations being needed (Guș et al., 2004; Luca and Nagy, 1999).

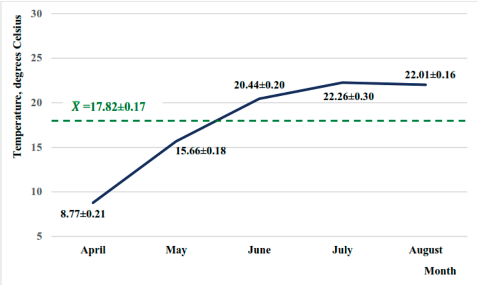

Figure 1. The mean monthly environmental temperature (°C), during vegetation period in experimental field, by 2021-2022

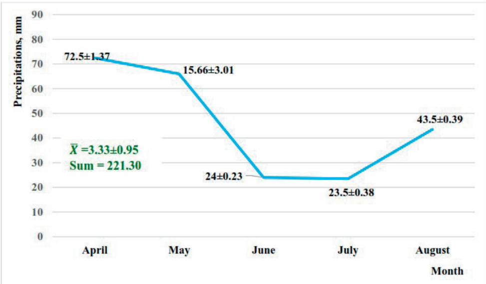

Figure 2. The mean monthly rainfall regimen (mm), during vegetation period in experimental field, by 2021-2022

In terms of means of dry matter (%), yield (t/ha), and sugar yield (t/ha), Vanghelis and Tesla hybrids show better performances compared to Gorilla and Penalty hybrids. Concerning dry matter, no significant differences are reported between treatments (irrigation and fertilization) within the same genotype. Lower values concerning dry matter content (9.51%-9.01%) are reported by Enchev and Bozhanska (2022) in an experiment involving sugar beet, fodder beet, and table beet. Even though better results are reported in dry matter content, sugar beet yield, and sugar yield, when NPK in ratio of 180-120-120 kg/ha (b_3) was administered, significant differences are emphasized only in sugar beet yields and sugar yields within each genotype between control and fertilization performed with NPK in ratio of 180-120-120 kg/ha (b_3) in both non-irrigated and irrigated experimental variants, at significance thresholds of 1%, and 5%, respectively (Table 2).

These results show, in overall, the positive influence of treatments consisting in irrigation and high NPK input on enhancing sugar beet yield, sugar yield and dry matter content. Lower yields (30.40-35.60 t/ha) compared with those obtained in our experiment are reported by Islamhulov et al. (2019) when different quantities of nitrogen inputs (N_{40} - N_{120}) were administered to Hercules sugar beet hybrid, and by Ijaz et al. (2023) in specific climatic conditions of Pakistan (6.80-13.20 t/ha), when organic fertilization was applied for two sugar beet genotypes.

Similar results concerning sugar beet yield (40.530-68.77 t/ha) are reported by Ahmad et al. (2012), but lower (with a single exception, the hybrid SD-PAK09/07) concerning sugar yield (4.44-7.08 t/ha) when testing NPK inputs 150-100-62.5, on 11 hybrids. Higher interval of sugar yield (6.80-13.20 t/ha), compared to our findings is reported by Ijaz et al. (2023) in specific climatic conditions of Pakistan, when organic fertilization was applied for California and Serenada sugar beet genotypes.

The ANOVA summary for the sugar beet yield shows that genotypes (G), and fertilization (F) have significant influence on probability level of 1%, also irrigation (I) and interaction between genotype and fertilization (G x F) influence the sugar beet yield, but at probability level of 5%. The interactions between genotype and irrigation (G x I), irrigation and fertilization (I x F), and all three factors (G x I x F). The genotype contribution participates to the total variance with 43.48%, the fertilization, and water supply contribution with 30.70%, and 11.25%, respectively, while G x F interaction with 10.65%. The other interactions had low contributions (Table 3). According to the same analysis (ANOVA) the sugar yield is significantly affected only by genotype, at probability level of 5%. The agricultural inputs (irrigation and fertilization), and interactions have no significant influences on sugar yields. The genotype accounted for 39.73% of variation, irrigation for 26.81%, fertilization for 24.25%, while the interactions have a low contribution (Table 4).

Curcic et al. (2018) also found that genotype has a significant influence on sugar yield even though is responsible only for 6.28-7.75% of variance, in an experiment with 5 sugar beet hybrids (including Tesla) within different environmental conditions.

Table 2. The yield, and sugar yield of studied sugar beets hybrids, 2021-2022

Hybrid	Experimental variant	n	Dry matter (%)		Yield (t/ha)		Sugar yield (t/ha)	
			$\overline{X} \pm s_{\overline{X}}$	CV%	$\overline{X} \pm s_{\overline{X}}$	CV%	$\overline{X} \pm s_{\overline{X}}$	CV%
Gorilla	a_1b_1	30	19.48ab±0.16	0.41	37.60abc±0.51	2.74	7.32ab±0.20	5.33
	a_1b_2	30	19.12ab±0.06	0.15	42.40abc±0.68	3.58	8.82ab±0.15	3.71
	a_1b_3	30	19.49ab±0.03	0.09	45.00abc±0.71	3.51	9.08ab±0.09	2.12
	a_2b_1	30	19.49ab±0.15	0.38	38.54abc±0.46	2.42	7.94ab±0.18	4.28
	a_2b_2	30	19.60ab±0.05	0.12	43.00abc±1.05	5.45	9.58ab±0.17	3.93
	a_2b_3	30	19.90ab±0.07	0.18	45.40abc±0.68	3.34	9.86ab±0.07	1.70
Vanghelis	a_1b_1	30	22.08ab±0.11	0.26	58.24abc±0.35	1.26	8.42ab±0.30	7.18
	a_1b_2	30	22.12ab±0.06	0.14	63.00abc±0.71	2.51	9.15ab±0.25	5.83
	a_1b_3	30	22.49ab±0.03	0.08	64.60abc±1.03	3.56	9.65ab±0.21	4.89
	a_2b_1	30	22.29ab±0.10	0.25	59.96abc±0.32	1.11	8.96ab±0.18	3.90
	a_2b_2	30	22.60ab±0.05	0.11	65.40abc±0.51	1.73	10.06ab±0.13	2.73
	a_2b_3	30	22.80ab±0.04	0.11	66.60abc±0.68	2.28	10.84ab±0.13	2.66
Tesla	a_1b_1	30	20.68ab±0.12	0.29	56.00abc±0.32	1.18	7.90ab±0.13	3.13
	a_1b_2	30	20.84ab±1.75	4.25	61.30abc±0.54	1.96	9.36ab±0.13	3.08
	a_1b_3	30	21.40ab±1.86	4.55	63.70abc±0.54	1.89	9.46ab±0.11	2.65
	a_2b_1	30	20.22ab±0.15	0.37	57.70abc±0.54	1.95	8.63ab±0.14	3.36
	a_2b_2	30	21.79ab±0.07	0.17	63.02abc±0.44	2.58	9.81ab±0.11	2.45
	a_2b_3	30	21.98ab±0.14	0.34	64.40abc±0.93	3.22	9.88ab±0.06	1.32
Penalty	a_1b_1	30	17.70ab±0.15	0.39	42.90abc±0.33	1.58	6.82ab±0.24	6.97
	a_1b_2	30	18.16ab±0.08	0.21	47.60abc±0.51	2.40	7.94ab±0.25	7.10
	a_1b_3	30	18.64ab±0.15	0.37	48.72abc±0.81	3.72	8.12ab±0.11	2.94
	a_2b_1	30	19.14ab±0.10	0.26	43.68abc±0.53	2.49	7.26ab±0.36	9.67
	a_2b_2	30	19.34ab±0.08	0.20	48.88abc±0.34	1.55	8.70ab±0.20	5.20
	a_2b_3	30	19.78ab±0.08	0.20	50.80abc±0.80	3.52	9.24ab±0.15	3.72

$\overline{X}$ - mean; $s_{\overline{X}}$ – standard error of mean; CV% - coefficient of variation; *Different letters signifies differences at significance threshold of 0.05%.

Table 3. Summary ANOVA for sugar beet yield by 2021-2022 period

Source of variation	DF	SS	MS	F	% of SS
G	2	367.54	183.77	480.65**	43.48
I	1	95.13	47.56	105.63*	11.25
F	2	259.47	259.47	401.55**	30.70
G x I	4	10.35	2.59	8.68	1.22
G x F	5	90.02	18.01	94.25*	10.65
I x F	2	4.16	2.08	3.61	0.49
G x I x F	7	18.36	2.62	25.89	2.17
Error	18	98.68	5.48	-	-

G - genotype; I - irrigation; F; fertilization; SS - sum of squares; MS - mean squares; G - genotype; I - irrigation; F - fertilization; DF - degrees of freedom; *, ** - the significance levels at $p < 0.05$, and $p < 0.01$.

Table 4. Summary ANOVA for sugar yield by 2021-2022 period

Source of variation	DF	SS	MS	F	% of SS
G	2	102.35	51.18	57.26*	39.73
I	1	69.07	69.07	29.68	26.81
F	2	62.48	31.24	25.12	24.25
G x I	4	5.32	1.33	2.68	2.07
G x F	5	3.29	0.66	3.71	1.28
I x F	2	2.88	1.44	2.02	1.12
G x I x F	7	12.21	1.74	15.62	4.74
Error	18	23.54	1.31	-	-

SS - sum of squares; MS - mean squares; G - genotype; I - irrigation; F - fertilization; DF - degrees of freedom; * - the significance level at $p < 0.05$.

The correlations between the environmental temperature and precipitations on one hand, and on the other hand sugar beet yield or sugar yield, function of agricultural inputs (irrigation, fertilization) are positive and strong or moderate to strong, in all cases (Table 5).

According to PCA, we identify the result of reducing the genotypes and production traits, in their components, which describe the variability of dry matter, root yield, and sugar yield, in specific climatic conditions.

Four principal factors were identified: genotype, production, agricultural inputs, environmental factors (Table 6), but the total variability of both yield traits may be summarized in two components accounting for 42.24%, and 25.08% of variance, and correspond to genotype, and production (Figure 3).

Table 5. Correlation matrix between sugar beet yield (Y), and sugar yield (SY), environmental temperature, and precipitations, function of experimental treatment within each studied genotype, by 2021-2022 period

Issue	Gorilla hybrid					
	Ya_1b_1	Ya_1b_2	Ya_1b_3	Ya_2b_1	Ya_2b_2	Ya_2b_3
Temperature (°C)	0.871	0.864	0.859	0.814	0.876	0.837
Precipitations (mm)	0.625	0.653	0.699	0.668	0.614	0.700
	Vanghelis hybrid					
	Ya_1b_1	Ya_1b_2	Ya_1b_3	Ya_2b_1	Ya_2b_2	Ya_2b_3
Temperature (°C)	0.734	0.735	0.749	0.735	0.761	0.773
Precipitations (mm)	0.624	0.625	0.679	0.630	0.706	0.689
	Tesla hybrid					
	Ya_1b_1	Ya_1b_2	Ya_1b_3	Ya_2b_1	Ya_2b_2	Ya_2b_3
Temperature (°C)	0.778	0.719	0.780	0.818	0.802	0.783
Precipitations (mm)	0.648	0.670	0.655	0.696	0.667	0.605
	Penalty hybrid					
	Ya_1b_1	Ya_1b_2	Ya_1b_3	Ya_2b_1	Ya_2b_2	Ya_2b_3
Temperature (°C)	0.778	0.752	0.685	0.804	0.766	0.758
Precipitations (mm)	0.629	0.598	0.616	0.594	0.770	0.739
	Gorilla hybrid					
	SYa_1b_1	SYa_1b_2	SYa_1b_3	SYa_2b_1	SYa_2b_2	SYa_2b_3
Temperature (°C)	0.754	0747	0.721	0.811	0.752	0.690
Precipitations (mm)	0.617	0.661	0.620	0.635	0.627	0.563
	Vanghelis hybrid					
	SYa_1b_1	SYa_1b_2	SYa_1b_3	SYa_2b_1	SYa_2b_2	SYa_2b_3
Temperature (°C)	0.782	0.826	0.849	0.788	0.735	0.806
Precipitations (mm)	0.564	0.627	0.694	0.669	0.639	0.668
	Tesla hybrid					
	SYa_1b_1	SYa_1b_2	SYa_1b_3	SYa_2b_1	SYa_2b_2	SYa_2b_3
Temperature (°C)	0.732	0.807	0.790	0.784	0.835	0.889
Precipitations (mm)	0.560	0.640	0.611	0.570	0.550	0.601
	Penalty hybrid					
	SYa_1b_1	SYa_1b_2	SYa_1b_3	SYa_2b_1	SYa_2b_2	SYa_2b_3
Temperature (°C)	0.754	0.725	0.804	0.770	0.706	0.758
Precipitations (mm)	0.524	0.571	0.664	0.710	0.575	0.539

Table 6. The eigenvalues of correlation matrix

Nr. crt.	Eigenvalue	% Total-variance	Cumulative-eigenvalues	Cumulative (%)
1	28.96	39.14	28.96	39.14
2	18.85	25.47	47.81	64.60
3	13.68	18.48	61.48	83.08
4	12.52	16.92	74.00	100.00

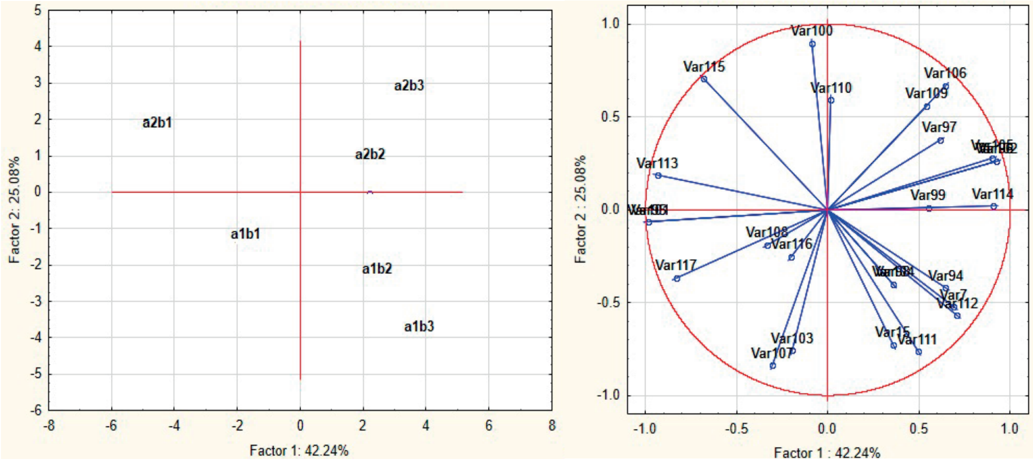

Figure 3. The PCA plot of the cases and variables on the factor plane

The first principal component (PC1) is related to all four genotypes. Irrigation (a_2) and culture supply with NPK in ratios of 60-40-40 kg/ha (b_2), and 180-120-120 kg/ha (b_3) contribute to PC1 increases, meaning these treatments have positive effects in all genotypes.

The same, irrigation (a_2) and no fertilization (b_1) and fertilization with NPK in ratios of 60-40-40 kg/ha (b_2), and 180-120-120 kg/ha (b_3) contribute to PC2 increases, meaning these treatments have positive effects for dry matter, root yield, and sugar yield. The negative direction of both PC1 and PC2 is related to lack of irrigation and fertilization.

Positive loadings for PC 1 (genotype) are identified for both environmental factors temperature (Var 7), and precipitations (Var 15), while for PC2 (productions), negative loadings are reported for the same factors (Figure 3). This suggest that low temperature and water inputs led to dry matter, root yield, and sugar yield decrease in all genotypes, and this finding is consistent with the results of the correlation matrix (Table 5).

CONCLUSIONS

To identify influence of environmental temperature, precipitations and agricultural key inputs (irrigation and fertilization) on analyzed sugar beet hybrid performances, dry matter, yield, and sugar yield, were quantified and correlated with environmental factors temperature and precipitations. The best results in terms of all analyzed production traits are reported in Vanghelis and Tesla hybrids when cultures are irrigated and fertilized with NPK in 180-120-120 kg/ha ratio. The roots yield is significantly influenced by genotype, irrigation, and interaction between genotype and fertilization, while sugar yield only by genotype. The correlations between the environmental temperature and precipitations, and analysed production traits, function of agricultural inputs (irrigation, fertilization) are positive, strong or moderate to strong. Four principal factors were identified, production, agricultural inputs, environmental factors, but the total variability may be summarized in two components accounting for 42.24% (genotype), and 25.08% of variance (production).

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RESULTS REGARDING VARIABILITY OF PRODUCTION AND SOME ELEMENTS OF PRODUCTIVITY TO FOUR PEA (*Pisum sativum*) GENOTYPES AT DIFFERENT SOWING TIMES

Elena BONCIU[1], Elena ROȘCULETE[1], Gabriela PĂUNESCU[2], Cătălin Aurelian ROȘCULETE[1], Rodica SOARE[1]

[1]University of Craiova, Faculty of Agronomy, 19 Libertatii Street, Craiova, Romania
[2]University of Craiova, SCDA Caracal, 106 Vasile Alecsandri Street, Caracal, Romania

Corresponding author email: rosculeta2000@yahoo.com

Abstract

The present paper presents the results obtained within a multifactorial experience at the Research and Development Station of the University of Craiova, SCDA Caracal, in the period 2021-2022. The biological material was represented by four pea varieties (Omega, Tiara, Favorit and Trinity), each of them being sown in two different seasons. The variability of production, plant height and some elements of productivity were monitored, such as: number of plants/m^2, number of pods/plant, length of pods, number of grains in pod and weight of 1000 grains. The results obtained showed a high variability, both in terms of production and productivity elements. Thus, the highest average production value was recorded by the Omega variety (4500 kg/ha) and the lowest one, Trinity (3635 kg/ha). The mass of 1000 grains had average values between 190.8 g (Omega) and 135.9 g (Tiara). Also, the ratio between plant weight and grains weight recorded average values from 1.74 (Tiara) and 1.59 (Favorit). These results confirm the very good suitability of the researched pea varieties for cultivation in the environmental and soil conditions of the South-West area of Oltenia.

Key words*: genotypes, pea, production, sowing times, variability.*

INTRODUCTION

Food security is approached as a multidimensional concept that takes into account both the need to ensure a sufficient agricultural supply, as well as ensuring the economic access of the population to food, the stability of the supply and the way of using food at the individual level (Amundson et al., 2015; Bonciu, 2019).

The responsibility for the availability of food falls mainly to agriculture, which must ensure a sufficient food supply to satisfy the food and nutritional needs of the population, and access to food is a problem that depends on the general situation of the economy, which ensures, through the level of the population's income, the purchasing power of food.

A systemic understanding of how agriculture, the economy and environmental health are interconnected is essential for identifying best practices available (Ahmad et al., 2019; Cotuna et al., 2022a, 2022b, 2022c). This is all the more necessary after some periods with serious consequences in the economy, like pandemic crisis which has put a lot of pressure on agriculture in a number of ways (Short et al., 2020).

The pea (*Pisum sativum*) is one of the most important legumes and is cultivated for its grains, which can be used for human consumption or animal feed, having an exceptional food and fodder value. It is highly appreciated for the high protein content in the green beans, but also carbohydrates, mineral salts and vitamins (Jain et al., 2014; Santos et al., 2019). The large amount of protein substances is remarkable, much higher (25%) compared to wheat (12.9%), as well as their special quality, given by the weight of essential amino acids: lysine, tryptophan, methionine and cysteine. The protein content is determined on the one hand by hereditary factors and on the other hand by culture conditions (Shanthakumar et al., 2022).

Regarding the forage quality of peas, the optimization of fodder rations with peas is more than timely, especially since, against the background of the prohibition of the use of antibiotics as growth promoters, the need has arisen to find fodder solutions for good health and good growth performance in animals, doubled by health protection of the consumer of animal products (Cola and Cola, 2021). In this respect, for each drug there is a waiting period for human protection. The waiting period is the time required for the antibiotic residue to reach concentrations below the tolerance levels (Cola and Cola, 2022). Maximum residue limits in European Union legislation guarantee consumer protection (Cola and Cola, 2019).

From the botanical point of view, pea is an annual herbaceous legume from the *Fabaceae* family, cultivated for its edible seeds and pods. The root system is strong and penetrates into the soil up to 40-60 cm deep, but, if the soil allows, it can penetrate up to one meter. The stem is hollow inside and slightly branched. The leaves are pinnately compound, alternately arranged, consisting of 2-3 pairs of leaflets, the last leaflet being transformed into a prison. Flowering takes place 30-50 days after sowing, and on a plant, flowering lasts 10-25 days. The fruit is a pod, straight or arched with a length between 3 and 12 cm. Pea seeds are yellow or green in the dry state.

The flowers, white or slightly purple-reddish, are arranged in the underside of the leaves, one for the early varieties and 2-5 for the late ones. The corolla has a butterfly shape. The stamens - the male reproductive organs - in peas are 9 in number through their filaments and the 10th free. In the middle of the flower is the gynoecium which is monocarpic - the carpels are modified leaves and adapted to the function of multiplication.

Pea plants are naturally self-pollinating. In self-pollination, pollen grains from anthers on one plant are transferred to stigmas of flowers on the same plant. The pollination is done directly, the pollen reaching the stigmas of the same flower, but cross-pollination is not excluded, when the pollen passes from one flower to another, through wind, insects, water and under human action (Bonciu et al., 2022).

The pea is considered a very good precursor plant for most vegetable species, as it leaves the land clean of weeds and especially rich in nitrogen, coming from the atmosphere due to the symbiosis that exists between the plants and the bacterium *Rhizobium leguminosarum* found on the nodules of the roots. Field peas alongside lentil (*Lens culinaris* Medik.) are pulse crops capable of forming a symbiosis with rhizobia for biological nitrogen fixation. As such, their inclusion in cropping systems is an important part of efforts to improve the sustainability of agriculture and decrease the reliance on nitrogen chemical fertilizers (Bourgault et al., 2022; Jiang et al., 2020; Pecetti et al., 2019).

Pea is a drought-resistant plant, but requires higher amounts of water during flowering to grain formation (Bourion et al., 2002; Larmure and Munier-Jolain, 2019).

The cultivation technology is generally simple. Peas are grown by sowing directly in the field. The most expensive work is the repeated harvesting of the green pods, which requires a lot of labor, mechanical harvesting being only partially solved for now. Garden pea culture requires some of the lowest production costs in the vegetable sector, as the technology allows full mechanization of the work (Sarūnaitė et al., 2022).

Expectations and forecasts for food security and agriculture worldwide indicate a slowdown in the general growth rate of agricultural production in the medium and long term, with the growth of agricultural production being focused, in the future, on developing countries. Food demand will continue to grow in the coming years as the world's population will increase considerably, and climate change will begin to put more and more pressure on water and soil resources.

Peas are one of the sustainable green crops, because they improve the quality of the soil, thus reducing the use of chemical fertilizers or fertilizers (Annicchiarico and Filippi, 2007).

MATERIALS AND METHODS

The demographic explosion and the current climate changes make future food security and safety dependent on the sustainability of soil fertility and the provision of water and energy resources, scientific research contributing to finding new sources and means leading to the sustainable provision of food needs. In this context, the present paper presents the results obtained within a polyfactorial experiment with four varieties of peas sown in two different times, at the Research Station of the University of Craiova, SCDA Caracal, in the period 2021-2022. The biological material was represented by four pea varieties (Omega, Tiara, Favorit and Trinity), each of them being sown in two different seasons (Figure 1).

Variability of production, plant height and productivity elements such as: number of plants/m^2, number of pods/plant, length of pods, number of grains per pod and mass of 1000 grains were monitored. For each of these indicators, the coefficient of variability (CV%) was calculated, using the Excel program, as the ratio between the standard deviation value and the average value.

The experiment was carried out on a typical argic (non-carbonic) chernozemic soil, with a well-defined profile and insignificant differences regarding the physical, hydric and chemical properties. The main characteristics of the soil were the following: humus content: 2.2%; total nitrogen: 104 ppm; phosphorus: 47 ppm; potassium: 244.5 ppm; pH: 5.4.

RESULTS AND DISCUSSIONS

The climatic conditions recorded during the entire vegetation period allowed obtaining optimal harvests. The temperatures during the growing season of the pea crop from 2022 were close to normal, in February registering +3.3^{0}C (Table 1).

Table 1. The evolution of the main climatic factors during the vegetation period of the pea genotypes tested at S.C.D.A. Caracal (2022)

Specification		I	II	III	IV	V	VI	TVP
T (°C)	Mmin	-9.2	-9.8	-8.5	-0.5	3.5	12.2	
	Mmax	15.5	17.4	22.5	26	32	38.6	
	Mavr	2.0	4.1	4.5	11.1	18.2	23	
	N	-1.3	0.8	6	12	17.7	21.6	
	Diff ± N	+0.7	+3.3	-1.5	-0.9	+0.5	+1.4	
Pp (mm)	Mtotal	19.2	4.8	13.2	77.8	44.6	14.2	173
	Mltavr	30.8	26.3	34.2	47.8	58.6	69.7	267.4
	Diff± N	-11.6	-21.5	-21	+30	-14	-55.5	-93.6
RH (%)	Avr	83.9	74.2	67.6	77.2	68	69	
	Min	59.8	43	37.8	44.4	31.5	32	

T - Temperature; Pp - Precipitations; RH - Relative air humidity; Mmin - Monthly minimum; Mmax - Monthly maximum; Mavr - Monthly average; N - Normal; Diff - Difference; Mtot - Monthly total, Mltavr - Multiannual average; Avr - Average; Min - Minimum; TVP - Total vegetation period.

Deviations of the average monthly temperatures compared to the multi-year average were also recorded in the months of January, March, April, May and June, but close together. These values led to an optimal development of the plants. Regarding precipitation, in the period January 2022-June 2022, 173.8 mm of precipitation was recorded, 93.6 mm less than the multiannual average (267.4).

The precipitation recorded in January and February was in deficit compared to the multi-year average, but contributed to a good preparation of the seed bed and an optimal emergence of the plants. In April, the amounts of precipitation were higher than the multiannual average by +30 mm and had a favourable influence on the development of crops. The relative humidity of the air oscillated on average between 68% in May and 83.9% in January.

The soil works consisted of plowing, harrowing, fertilizing with 100 kg/ha NPK 20-20-0 complex fertilizers and preparing the germination layer with the combiner. The four pea varieties were each sown in two different seasons, shown in Figure 1. The predecessor plant was maize.

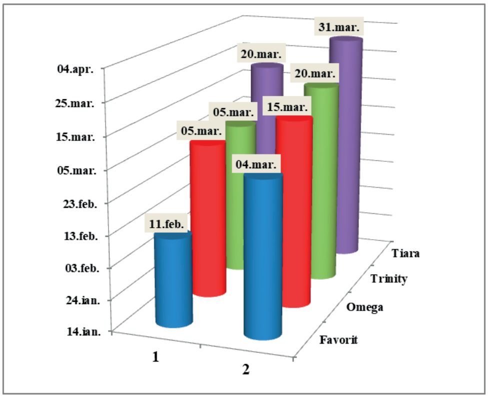

Figure 1. Sowing date in two different periods for each pea variety at SCDA Caracal (2022)

The width of each sown plot was 4 m, with a length of 100 m and an area of 400 m^2. Sowing depth: 5 cm, and the distance between rows: 12.5 cm. After the plants emerged, a herbicide work with Butoxone was carried out, respectively two phytosanitary treatments with the products Krima 20 SG (acetamiprid) and Decis Expert 100 EC (deltamethrin).

Figure 2 shows images from the experimental field.

Figure 2. Pea culture at SCDA Caracal

The phenological observations and biometric determinations carried out on the four pea varieties sown in the first season are shown in Table 2, respectively in Table 3 for the second sowing season. Thus, in the first sowing period, the number of plants/m^2 was between 120 (Omega and Tiara) and 125 (Favorit and Trinity); the height of the plants had values between 49.3 cm (Trinity) and 53.8 (Favourite); the number of pods/plant was between 5 (Tiara and Trinity) and 7 (Favorite) and the number of grains in the pod was between 6 (Omega, Tiara, Trinity) and 7 (Favorite).

The weight of the grains per plant recorded values between 5.11 g (Tiara) and 6.20 g (Omega), while the mass of 1000 grains was between 137 g (Tiara) and 190.8 (Omega).

Table 2. Phenological observations and biometric determinations to peas tested at SCDA Caracal (Sowing season I)

Variety	NP /m^2	BT	PH (cm)	NPP	PL (cm)	NGP	GW (g)	MMB (g)
Omega	120	15.05	50.3	6	4.8	6	6.20	190.8
Tiara	120	18.05	52.1	5	5.6	6	5.11	137.0
Favorit	125	4.05	53.8	7	5.7	7	6.14	165.2
Trinity	125	16.05	49.3	5	5.2	6	5.42	181.3
CV(%)	2.3	-	3.9	16.6	7.7	8.0	9,4	13.9

NPm^2 - No.plants/m2; BT - Bloomng time; PH - Plant height; NPP - No.pods/plant; PL - Pods length; NGP - No.grains/pod; GW - Grain weight/plant; MMB - Mass of 1000 grains; CV - Coefficient of variability (%).

Table 3. Phenological observations and biometric determinations to peas tested at SCDA Caracal (Sowing season II)

Variety	NP /m^2	BT	PH (cm)	NPP	PL (cm)	NGP	GW (g)	MMB (g)
Omega	120	21.05	56.2	5	4.9	6	6.11	180.2
Tiara	120	8.05	56.3	4	5.7	7	4.88	135.9
Favorit	125	11.05	55.7	6	6.1	6	5.79	163.5
Trinity	125	7.05	53.5	5	5,5	5	5.17	177.3
CV (%)	2.3	-	2.4	16.3	9.0	13.6	10.2	12.3

NPm^2 - No.plants/m2; BT - Bloomng time; PH - Plant height; NPP - No.pods/plant; PL - Pods length; NGP - No.grains/pod; GW - Grain weight/plant; MMB - Mass of 1000 grains; CV - Coefficient of variability (%).

In the second sowing season, the plant height of the four pea varieties was between 53.5 cm (Trinity) and 56.3 (Tiara); the number of pods/plant was between 4 (Tiara) and 6 (Favourite), and the number of grains in the pod was between 5 (Trinity) and 7 (Tiara). The weight of the grains per plant recorded values between 4.88 g (Tiara) and 6.11 g (Omega), while the mass of 1000 grains was between 135.9 g (Tiara) and 180.2 (Omega).

Analysing the obtained results, a high variability was found, both in terms of production and productivity elements. Thus, the highest average production value between the two sowing seasons was recorded by the Omega variety (4500 kg/ha), followed by Favorit (3900 kg/ha), Tiara (3700 kg/ha) and Trinity (3635 kg/ha) (Figure 3). Also, the ratio between plant weight and grains weight recorded average values from 1.59 in the Favorit variety to 1.74 in the Tiara variety (Figure 4).

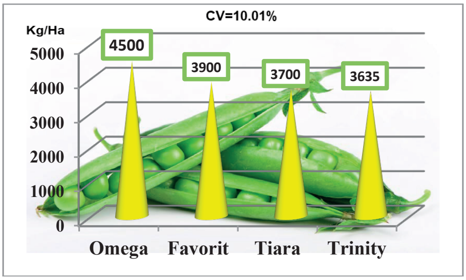

Figure 3. Variability of production for peas (average of two different sowing seasons) to SCDA Caracal
CV - Coefficient of variability (%)

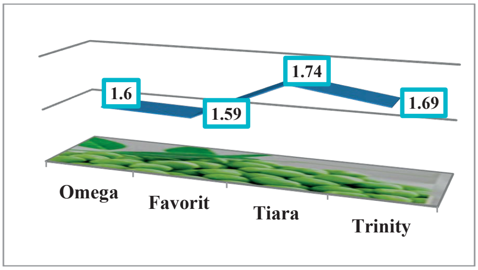

Figure 4. Variability of the plant weight and grain weight ratio in peas grown at SCDA Caracal

Sirohi et al. (2006) reported high phenotypic and genetic coefficients of variability to pea for plant height, number of pods per plant and grains yield per plant indicating greater scope of selection for these traits. Similarly, the results obtained within the SCDA Caracal experiences confirm the high variability of production and productivity elements.

Reduced seed abortion and improved of seeds number per pod is correlated with pea yield in both stressful and favourable conditions, as other authors have suggested (Kumar et al., 2013; Sadras et al., 2019; Sibhatu et al., 2016; Smith et al., 2005). In order to make pea crops more efficient, it is necessary to use high-performance combines, which perform simultaneously with the harvesting and threshing of the green pods, directly in the field.

The grains yield of pea depends on a several factors, such as genotype, growing conditions and genotype and environment interaction (Carlson-Nilsson et al., 2021). In our experiment, the variability of the grain yields of pea tested genotypes was high. However, the climate changes have a negative impact on crop productivity globally, including leguminous crops; they also have an indirect impact on biotic constraints, which may lead to the invasion of weeds, pests, and pathogens in previously unaffected areas (Paraschivu et al., 2022, 2021; Cotuna et al., 2021; Velea et al., 2021). It is necessary, from this point of view, to introduce into the culture technologies a variety of cultivation resistant to diseases and pests, with high productive potential and increased yield of grains/pods (Namatsheve et al., 2020).

The results obtained in this study confirm the very good suitability of the four pea varieties tested at SCDA Caracal for sustainable cultivation in the environmental and soil conditions of the experimented area.

CONCLUSIONS

The results obtained showed a high variability, both in terms of production and productivity elements. Thus, the highest average production value between the two sowing seasons was recorded by the Omega variety (4500 kg/ha), followed by Favorit (3900 kg/ha), Tiara (3700 kg/ha) and Trinity (3635 kg /ha).

The mass of 1000 grains also showed high variability, with average values between 190.8 g (Omega) and 135.9 g (Tiara). On the other hand, the ratio between plant weight and grain weight recorded average values from 1.59 in the Favorit variety to 1.74 in the Tiara variety.

The obtained results confirm the very good suitability of the four pea varieties for sustainable cultivation in the environmental and soil conditions of the South-West area of Oltenia. We consider, on the other hand, that considerable profits can be obtained by using very early varieties or by establishing crops in autumn, in this way realizing extra-early pea productions, which are valued at high prices.

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SEED GERMINATION AND EARLY SEEDLING GROWTH OF PEA (*Pisum sativum* L.) IN RESPONSE TO SEED PRIMING

Dorina BONEA, Mihai CICHI

University of Craiova, Faculty of Agronomy, 19, Libertatii Street, Dolj County, Romania

Corresponding author email: mihaicichi@yahoo.com

Abstract

The early growth ability of plants strongly influences their growth and yields. Seed priming is widely used for better germination and emergence of plants both under stressful and non-stressful conditions. The purpose of this study was to determine the effects of seed priming on some growth parameters of Pisum sativum seedlings and to identify the most suitable priming techniques. For this reason, a laboratory experiment was carried out in randomized blocks with three repetitions, in which pea seeds were primed for 6 hours with distilled water and with two concentrations and combinations of salicylic acid and calcium carbonate (CaCO3). The treatment non-priming was used as a control. The results showed that the priming treatments had significant effects on all studied parameters ($p<0.05$). Seed priming with both concentrations of calcium carbonate (1 g/l and 2 g/l) and hydro-priming (with water) showed the best priming effects on root and shoot length, root and shoot fresh weight, and seedling vigour index. Therefore, these priming techniques, which are cheap and ecological, can be used by farmers for the successful establishment of pea crops.

Key words: *calcium carbonate, germination seed, pea, salicylic acid, seedlings.*

INTRODUCTION

Pea (*Pisum sativum* L.) is one of the most important food legumes worldwide due to its multiple uses: forage dry matter, green grains, green fodder, flour, straw and green manure. It is a rich source of protein because 100 grams of green pea (grains) contain 6.55 g of protein, 0.84 g of lipids and 0.92 g of mineral salts (Roman et al., 2011).

In Romania, pea seeds are generally sown in autumn or early spring, thus being exposed to cold stress. This low-temperature stress during germination can lead to suppression of germination and low seedling vigour. Also, other factors can lead to a decrease in pea production, such as fungal infections, the small number of nodules, the slow process of grain filling, and to overcome these problems, Gour et al. (2019) recommend the seed priming method.

The decisive factors that ensure successful crop establishment are uniform germination and seedlings vigour, because these factors contribute to the uniform growth and maturity of the plants and finally to a high yield (Finch-Savage & Bassel, 2016; Drăghici et al., 2021; 2022). Therefore, the main purpose of the farmer is to stimulate some essential stages in ensuring high yields by improving germination and seed vigour (Karim et al., 2020).

Priming is a simple method used for rapid germination and emergence that is applied before sowing and consists of partially hydrating the seeds with different priming agents, followed by drying the seeds. According to Elkoca (2014), the priming method permits partial seed hydration so that pre-germination metabolic activities proceed but primary root protrusion is prevented. This is a simple, low-risk, low-cost technique that can be useful for farmers (Dessalew et al., 2022).

Notable results for various plant species regarding improving the germination and seedling emergence by different priming agents (i.e. polyethylene glycol, abscisic acid, glycinbetaine, gibberellic acid, salycilic acid, KNO_3, etc.) or plant extracts, were reported previously (Hussain et al., 2015; Bonea, 2016; Kumari et al., 2017; Bonea, 2018; 2020; Jia et al., 2020).

However, the success of seed priming depends on several factors such as priming agents, duration of priming and seed condition. Therefore, optimizing the priming technique for a specific crop is an area worth exploring to improve crop productivity under various environmental conditions (Karim et al., 2020).

Some previous studies have reported that hydro-priming is an agronomically efficient procedure with the potential to increase tolerance to many environmental stress conditions (Plazek et al., 2018; Rhaman et al., 2021).

Salicylic acid has an important role in regulating many plant processes increasing tolerance to abiotic stress (Noreen et al., 2009; Ma et al., 2017).

Many studies found that seed priming with salicylic acid improves the germination and viability of seeds in wheat (Soare et al., 2015), rice (Shatpathy et al., 2018), faba bean (Anaya et al., 2018), okra (Rhaman et al., 2021).

Calcium is an essential nutrient for plant growth, having various structural roles in cell walls and membranes and in key enzyme activities (White & Broadley, 2003). Calcium also has a central role in many defense mechanisms that are induced by stress (Cousson, 2009).

Several studies have reported that $CaCl_2$ (as a calcium source) used as a seed priming agent in different plant species stimulated germination and vegetative growth under drought or salinity conditions (Aroubandi, 2016; Kaczmarek et al., 2017; Gao &Yan, 2020), but little information exists concerning the effect of $CaCO_3$ on seed germination and seedling growth.

In the context of the previously mentioned, this study was carried out to evaluate the effects of some priming treatments on growth parameters of pea seedlings.

MATERIALS AND METHODS

Plant material

The pea seeds (cv. Kelvedon) were purchased from Mefim Agro SRL (Craiova, RO).

An experiment was carried out at the Faculty of Agronomy, Breeding of plants laboratory in 2022. The seeds were sterilized with 5% sodium hypochlorite solution for five minutes and rinsed with distilled water, then dried at 22±2°C for 24 hours.

Seed Priming Protocol

For priming, pea seeds were subjected to hydro-priming (distilled water) and priming with 1 g/l and 2 g/l of salicylic acid (SA); 1 g/l and 2 g/l of calcium carbonate ($CaCO_3$) and combinations of 1 g/l and 2 g/l $CaCO_3$ + 1 g/l and 2 g/l SA for 6 hours, then dried to the original moisture content.

Non-priming treatment was used as a control.

Germination test

Three replicates, each with 30 seeds for each treatment variant, were germinated on two layers of filter paper in plastic pots. 5 ml of distilled water was added to each pot for seven days. The plastic pots were kept at room temperature (22±2°C).

Data Collection

Seed germination was recorded every 24 h for 7 days, and seeds were considered germinated when their radicle was at least 2 mm long.

The following germination and seedling vigour parameters were measured using the following equations:

Germination percentage (%) = (Nr. germinated seeds/Total nr. of seeds) × 100

The seedling vigour index (SVI) was calculated according to Abdul-Baki & Anderson (1973) as:

Seedling vigour index (SVI) = Seedling length × G (%),

Where G, is the germination percentage

Seven days after seed priming, ten normal seedlings were randomly selected from each plastic casserole to record seedling growth parameters, i.e. root length and shoot length, and their fresh weight.

The length was measured with a ruler and the fresh weight was determined using a precision balance.

Statistical analysis

Statistical analysis was performed using the analysis of variance (ANOVA), and treatment means were compared using Duncan's multiple comparison tests at a 5% level of probability.

RESULTS AND DISCUSSIONS

According to the results, all studied parameters (germination percentage, root length, shoot length, root fresh weight, shoot fresh weight, and seedling vigour index were significantly affected by different priming treatments (Table 1).

Significantly higher germination percentages were recorded priming pea seeds with calcium carbonate in both concentrations of 2 g/l and 1 g/l (98% and 95%), in the treatment non-priming (95%) and in the treatment with hydro-priming (87%), and a significantly lower germination percentage was recorded in the treatment with 2 g/l salicylic acid (12%), compared to other treatments (Figure 1).

Table 1. ANOVA table for effects of priming treatments on studied parameters

Parameter	MS	F-value
Germination percentage	2928.420	36.99*
Root length	5.779	8.47*
Shoot length	10.053	16.38*
Root fresh weight	0.007	15.87*
Shoot fresh weight	0.015	12.31*
Seedling vigour index	354910.100	17.19*

* - Significant at 5% level of significance; MS - Mean sum of squares.

Anaya et al. (2018) pointed out that a lower concentration of salicylic acid can improve seed germination by increasing gibberellic acid biosynthesis. At the same time, a high concentration of salicylic acid can stop seed germination as a result of enhancing ABA synthesis (Wu et al., 1998) or can increase oxidative stress that leads to the degradation of leaf pigments (Rhaman et al., 2021).

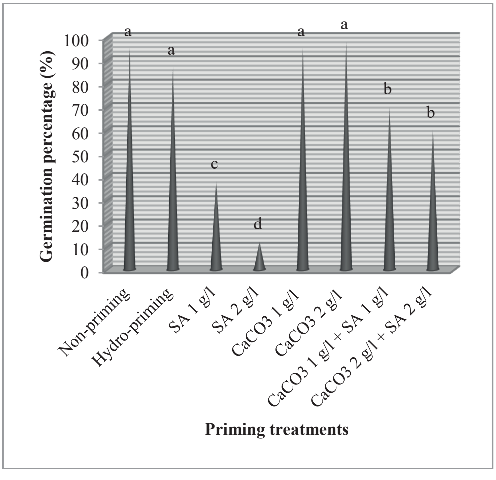

Figure1. Germination percentage of pea seeds in different priming treatments. Different letters show significant differences at the 5% probability level

Significantly, maximum root length (4.76 cm) was recorded in seed primed with 1 g/l calcium carbonate as compared to other priming treatments, followed by hydro-priming (3.97 cm) and priming by 2 g/l calcium carbonate (3.81 cm). The lowest root lengths (0.83 and 1.27 cm) were observed when priming seeds with both concentrations of salicylic acid (1 and 2 g/l) (Figure 2).

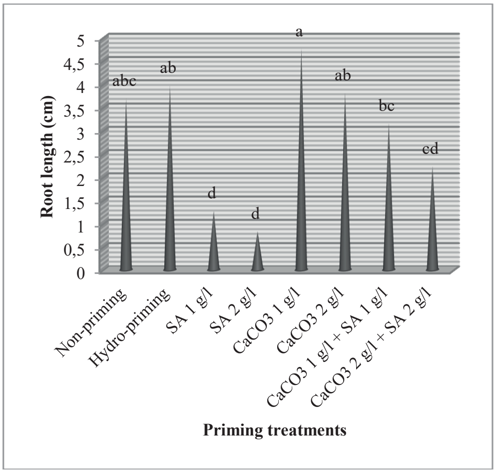

Figure 2. Root length of pea seed in different priming treatments. Different letters show significant differences at the 5% probability level

Significantly highest shoot lengths were recorded in the seed primed with 2 g/l calcium carbonate (4.74 cm), hydro-priming (4.24 cm) and priming with 1 g/l calcium carbonate (4.23 cm). Shoot lengths significantly lower, compared to other priming treatments, were recorded in the seed priming with 2 g/l salicylic acid (0.33 cm), the priming with 1 g/l salicylic acid (0.59 cm) and the priming with the combination of 2 g/l salicylic acid + 2 g/l calcium carbonate (0.63 cm) (Figure 3).

For the fresh weight of the root, significantly high values (0.173 g) were recorded in the treatment with 1 g/l calcium carbonate (0.173 g) followed by the treatment with 2 g/l calcium carbonate (0.129 g), and the lower value was recorded in the treatment with 2 g/l salicylic acid (0.031 g) compared to other treatments (Figure 4).

Significantly higher shoot fresh weights were recorded in seed primed with both concentrations of 1 and 2 g/l calcium carbonate (0.182 g and 0.165 g, respectively) and in hydro-priming treatment (0.158 g), whereas lower values were found in the non-priming treatment and in other priming treatments (Figure 5).

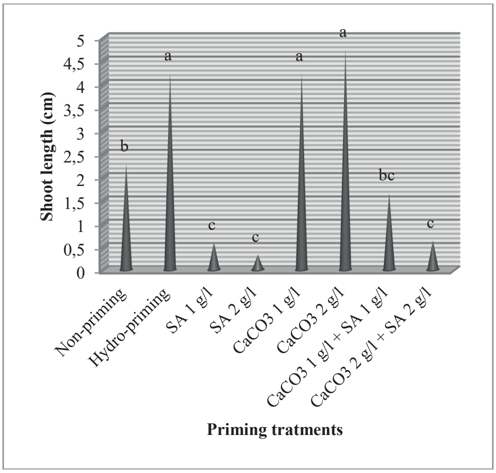

Figure 3. Shoot length of pea seed in different priming treatments. Different letters show significant differences at the 5% probability level

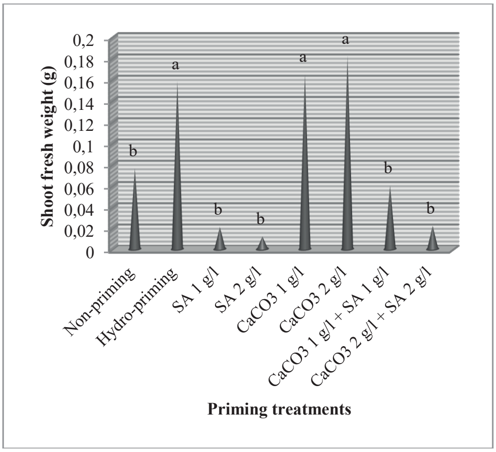

Figure 5. Shoot fresh weight of pea seed in different priming treatments. Different letters show significant differences at the 5% probability level

According to Yanglem et al. (2016), this could be due to the removal of harmful substances such as inhibitory hormones and higher seed leachates at the time of seed invigoration.

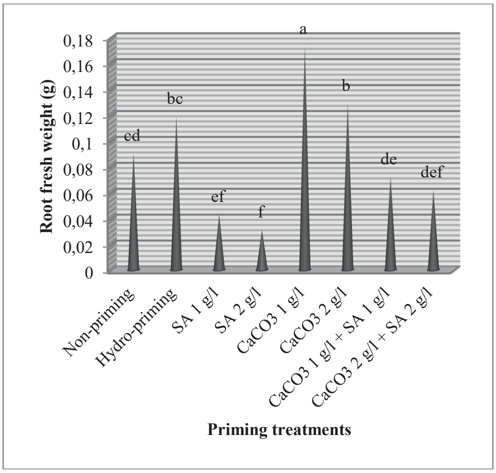

Figure 4. Root fresh weight of pea seed in different priming treatments. Different letters show significant differences at the 5% probability level

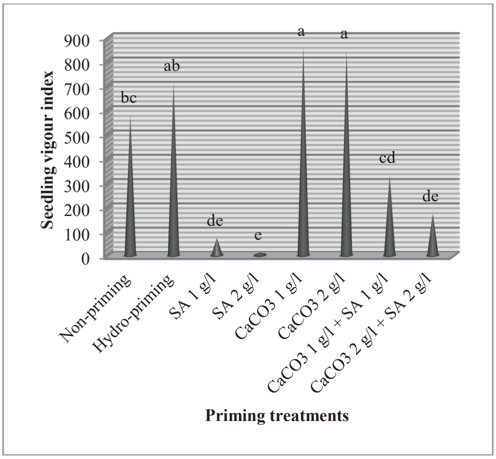

Figure 6. Effect of different seed priming treatments on seedling vigour index. Different letters show significant differences at the 5% probability level

Significantly, maximum seedling vigour indices were recorded in seed priming with calcium carbonate in both concentrations of 1 and 2 g/l (855.3 and 843.5, respectively) followed by hydro-priming treatment (711.0) as compared to other treatments. The lowest seedling vigour index was observed in priming with 2 g/l salicylic acid (13.8) (Figure 6).

Hao et al. (2020) reported that high seed vigour would determine the potential for rapid and uniform seed emergence and can increase yield by up to 20%.

A positive effect of calcium carbonate was also observed by Sadak and Talaat (2021) who reported that seed priming with 20 mg/l and 40 mg/l calcium carbonate significantly promoted wheat growth under normal and stressed conditions. Belur et al. (2010) reported that higher seed quality parameters in soybean could be obtained by pre-sowing seed treatment of 1% calcium carbonate, compared to hydro-priming treatment.

Other studies have shown that priming wheat seeds with combinations of Ca^{2+} and salicylic acid increased the production of phenolics compounds, thus leading to better seed vigour (Yücel & Heybet, 2016).

CONCLUSIONS

The results of the present experiment showed that different priming treatments had significant effects on the germination percentage and seedling growth of pea.

Priming with both concentrations of calcium carbonate (1 g/l and 2 g/l) and hydro-priming showed the most significant effects on root and shoot length, root and shoot fresh weight, and seedling vigour index.

These priming techniques, which are cheap and ecological, can be used by farmers to improve the growth of pea seedlings when the plants are not growing properly as they expected.

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STUDY ON SUNFLOWER PRODUCTION ESTIMATION BASED ON AERIAL IMAGES (UAV)

Ciprian BUZNA[1], Marinel Nicolae HORABLAGA[1, 2], Florin SALA[1, 2]

[1]Agricultural Research and Development Station Lovrin, Lovrin, 307250, Romania
[2]University of Life Sciences “King Mihai I” from Timisoara, 119 Calea Aradului Street, 300645, Timisoara, Romania

Corresponding author email: florin_sala@usab-tm.ro

Abstract

The study used the technique based on aerial images (UAV) to estimate the production of the sunflower crop, hybrid P64LE25. The experiment was organized within SCDA Lovrin, Timis County, Romania, in the 2021-2022 agricultural year, under the conditions of a chernozem type soil, non-irrigated system. The images were taken with the drone (DJI Phantom 4), at variable heights (H, 3 to 50 m). From the analysis of the digital images, the values of the RGB color parameters were obtained. The distribution of the RGB data series was of normal type (r = 0.950 for R, r = 0.960 for G and r = 0.965 for B). Very strong correlations were recorded between color parameters (r = 0.994 for R and G, r = 0.954 for R and B, r = 0.948 for G and B), and weak correlations between RGB and H. From PCA, PC1 explained 97.698% of variance, and PC2 explained 2.1027% of variance. Regression analysis facilitated the prediction of sunflower production based on RGB values, under statistical safety conditions (p<0.001; RMSEP = 1.7907 in relatin to R and G; RMSEP = 4.5869 in relation to R and B; RMSEP = 3.6978 in relation to G and B).

Key words*: imaging analysis, modeling, production estimation, sunflower, UAV images.*

INTRODUCTION

The sunflower is a plant of interest for seed production, as a resource for the food industry, the pharmaceutical industry, as a honey plant, for biodiesel, but also in animal feed (Adeleke and Babalola, 2020; Giannini et al., 2022).
Although today the sunflower has multiple uses, initially, for a long period of time, it was used for ornamental and medicinal purposes (Giannini et al., 2022).
Within agricultural crops, sunflowers are important in the structure of crop plants, in crop rotations, for an integrated management of crops and offer multiple ecosystem benefits (Debaeke et al., 2017)
The sunflower is also considered to be an "ecological crop", as a result of the lower amounts of nitrogen required, the high tolerance to thermal and water stress (reduction of irrigation), as well as the reduced pesticides (Debaeke et al., 2017a, 2017b).
Against the background of climate changes and in the context of geopolitical scenarios, it is considered important for the perspective to improve sunflower genotypes with tolerance and resistance to stress factors (biotic, abiotic), better/updated agronomic practices, exploration and expansion of new cultivation areas (Debaeke et al., 2017a; 2017b; Duca et al., 2022; Giannini et al., 2022).
In the context of the ideas of expanding the cultivation areas for sunflower, including through the valorization of some lands considered marginal, as well as for the improvement, updating and adaptation of culture technologies, different inputs have been tested and studied for the culture of sunflower, as a biocar, nanoparticles, etc. (Pîrvulescu et al., 2015; Chiaramonti and Panoutsou 2019; Kornarzyński et al., 2020).
The evaluation and monitoring of crops based on image analysis (satellite, aerial, terrestrial images) is of high importance and has been highlighted in various studies (Govedarica et al., 2015; Sala et al., 2020; Omia et al., 2023).
Techniques based on remote sensing, as well as different models obtained from spectral information, were used in the study of sunflower culture to evaluate and characterize the dynamics of vegetation stages (Herbei and Sala, 2015), to evaluate the water regime and the

carbon budget (Pique et al., 2020), or for the estimation of sunflower production (Dicu et al., 2021; Amankulova et al., 2023).

As a result of specific facilities, the technique based on UAV images was used in the study of sunflower culture to evaluate weed management (López-Granados et al., 2016), plant lodging and associated effects, quantitative and qualitative yield losses (Song et al., 2020; Li et al., 2021), obtaining information for precision agriculture, regarding aerial biomass, N content, yield (Vega et al., 2015).

The present study used the technique based on UAV images in the analysis, characterization of sunflower crop and prediction of sunflower yield, based on a set of images taken at variable heights and the generation of prediction models in relation to RGB color parameters obtained from digital images.

MATERIALS AND METHODS

The study took place within ARDS Lovrin, Romania, and used the technique based on aerial images (UAV) to analyze, characterize a sunflower crop and predict seed production according to spectral information (RGB) obtained from digital images.

The sunflower hybrid P64LE25 (Corteva) was cultivated under non-irrigated conditions, on a medium-fertility chernozem soil. Sowing was done on April 13, 2022. Fertilization was done with complex fertilizers (15:15:15) 200 kg ha^{-1}, and granular ammonium nitrate 250 kg ha^{-1} (a fraction of AAG applied in vegetation, stage 6 leaves). Weed control was ensured by herbicides with a mono and dicotyledonous spectrum (Spectrum, 1.5 L ha^{-1}, pre emergent application; Fusilade 1.5 L ha^{-1}, Express 30 g ha^{-1}, post emergent application). Plant protection against diseases was done with appropriate products (eg. Retengo 1 L ha^{-1}, applied on May 31). The crop had a normal vegetative course, but the climatic conditions specific to the year 2022 had an effect on the production. At physiological ripening, seed production was harvested, with an average value per plot of 3650 kg ha^{-1}.

To carry out the study, a logical flow diagram was designed (Figure 1).

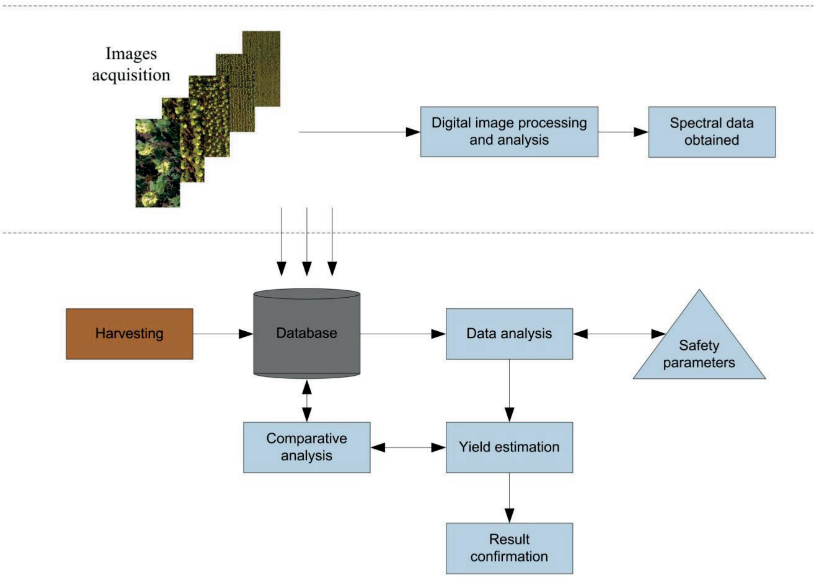


Figure 1. Logical flow diagram of the study process

In the vegetation stage of the crop, 7 BBCH Code (Meier 2001), aerial images were taken with the drone (DJI Phantom 4) at variable heights from the ground level, between 3 and 50 m, and resulted in 16 series of images (16 experimental variants, T1 to T16). The images, digital format, jpeg, were analyzed to obtain the RGB spectral values. The obtained data were analyzed to evaluate the distribution of the data series, the presence of variance and statistical reliability, the interdependence between RGB color parameters, the distribution and the degree of similarity between variants.

Regression analysis was used to estimate production based on RGB parameters (in different combinations). The safety level of the results was assessed based on appropriate statistical safety parameters.

The results obtained, in the form of series of values of the RGB color parameters, and data obtained through calculations and were analyzed by appropriate methods (Hammer et al., 2001; Wolfram Alpha 2020).

RESULTS AND DISCUSSIONS

Numerical information for RGB color parameters was obtained from the analysis of aerial images taken with a drone (UAV). In relation to the height of taking the images, the variation of the values of the color parameters was found, R = 83.12-94.39±0.91, G = 77.75-86.78±0.76, respectively B = 33.85-39.32±0.45 (Table 1).

Table 1. The values of the RGB color parameters in relation to the UAV image capture height, the sunflower crop, the P64LE25 hybrid

Trial	H (m)	R	G	B
T1	3	91.85	84.57	37.51
T2	4	92.58	85.75	37.29
T3	5	91.11	84.14	37.72
T4	6	92.1	85.49	38.55
T5	7	93.27	86.72	39.13
T6	8	83.12	77.75	33.85
T7	9	84.66	78.975	34.37
T8	10	86.2	80.2	34.9
T9	15	83.36	77.8	34.94
T10	20	87.31	80.52	35.76
T11	25	89.32	82.47	36.52
T12	30	90.52	83.18	37.54
T13	35	91.42	84.13	38.39
T14	40	91.48	84.44	38.9
T15	45	93.35	85.84	39.04
T16	50	94.39	86.78	39.32
SE		±0.92	±0.77	±0.45

The series of values, for color parameters (RGB), resulting from the analysis of digital images, presented normal distributions, under statistical safety conditions (r = 0.950 for R, r = 0.960 for G, and r = 0.965 for B). Figure 2 shows the distribution of the data series for the analyzed RGB parameters.

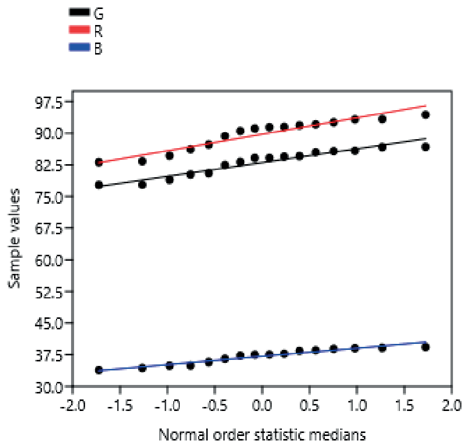


Figure 2. Distribution of RGB values in relation to the image acquisition height (UAV), sunflower crop, hybrid P64LE25

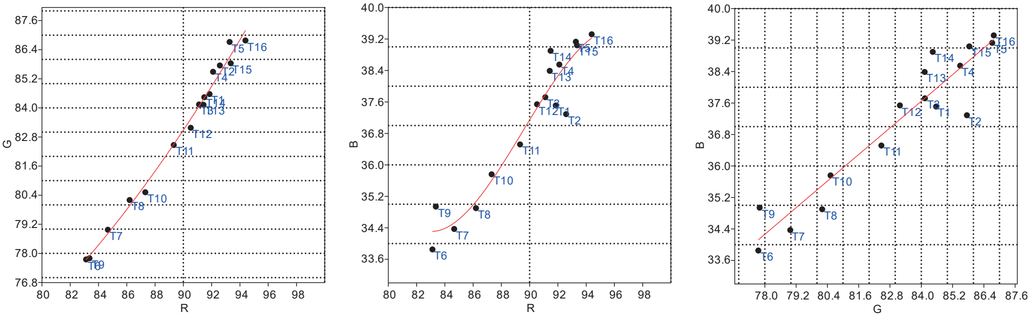
Very strong, positive correlations were recorded between RGB color parameters (r = 0.994 for R with G, r = 0.954 for R with B, and respectively r = 0.948 for G with B). Weak correlations were the result, from the analysis, between the RGB color parameters and the image capture height.

The interdependence relationship between color parameters in the characterization of the sunflower crop, the hybrid P64LE25, was evaluated by regression analysis. The interdependence relationship between R and G was described by a 2nd degree polynomial equation, equation (1), under conditions of $R^2 = 0.944$, $p < 0.001$, F = 635.02. The interdependence relationship between R and B was described by a polynomial equation of degree 3, equation (2) under conditions of $R^2 = 0.921$, $p < 0.001$, F = 46.739. The interdependence relationship between G and B was described by a linear equation, equation (3), under conditions of $R^2 = 0.898$, $p < 0.001$, F = 124.53. The graphic distribution of the interdependence of the identified color parameters, and the expressions of the obtained equations, is presented in Figure 3.

$$G = 0.0141 \cdot R^2 - 1.672 \cdot R + 119 \tag{1}$$

$$B = -0.004655 \cdot R^3 + 1.251 \cdot R^2 - 111.4 \cdot R + 3328 \tag{2}$$

$$B = 0.5638 \cdot G - 9.71 \tag{3}$$



(a) (b) (c)

Figure 3. The graphic distribution of the interdependence of the RGB color parameters in the description of the sunflower crop, the hybrid P64LE25; (a) G in relation to R, (b) B in relation to R, (c) B in relation to G

On the basis of PCA, correlation, the distribution diagram of the variants, represented by the image capture height (T1 to T16), was obtained in relation to RGB parameters, as biplot (Figure 4).

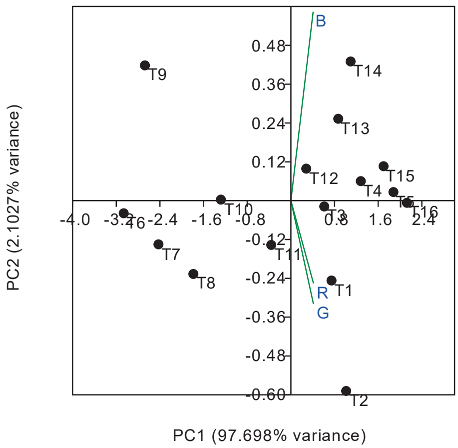


Figure 4. PCA diagram, correlation, regarding the distribution of variants in relation to RGB color parameters, sunflower crop, hybrid P64LE25

According to PCA, PC1 explained 97.698% of variance, and PC2 explained 2.1027% variance. It was found the independent positioning of some variants in relation to RGB parameters (T6, T7, T8, T9, T10 and T11) and the placement of the other variants associated with color parameters (T1 associated with R and G; T12, T13 and T14 associated with B; the other variants had an intermediate position between R, G, respectively B).

The cluster analysis led to obtaining a grouping of the variants based on similarity in relation to the values of the RGB color parameters. The dendrogram in Figure 5 was obtained, in which the variants (T1 to T16) were grouped based on similarity in relation to the values of the RGB color parameters. Two distinct clusters resulted, with several sub-clusters each.

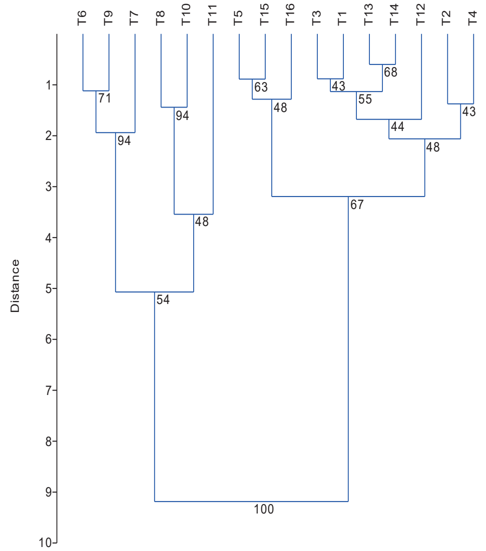


Figure 5. The cluster grouping based on the Euclidean distances of the variants, in the imaging analysis of the sunflower crop, hybrid P64LE25

A cluster C1 included the variants [(T6, T9) T7] within a subcluster C1-1 and the variants [(T8, T10) T11] within a sub-cluster C1-2, variants with position independent of RGB parameters. Within the C2 cluster, the other variants were grouped in two sub-clusters. Sub-cluster C2-1 included the variants [(T5, T15), T16], with an intermediate position, relative to RGB parameters.

Within the sub-cluster C2-2, variants (T1, T3) were associated in close relation with parameters R and G, variants [(T13, T14) T12 were associated in close relation with parameter B], and variants (T2, T4) were positioned associated, as a similar position in relation to RGB.
Based on the SDI values, the highest level of similarity was assessed between variants T13 and T14 (SDI = 0.5998), followed by variants T1 and T3 (SDI = 0.8812) and variants T5 and T15 (SDI = 0.8882) within the C2 cluster.
Within the C1 cluster, a high level of similarity was recorded between the variants (SDI = 1.1172), followed by the T8 and T10 variants (SDI = 1.4402).
Considering the level of safety regarding the distribution of the data series for RGB parameters, the distribution and association of the variants (T1 to T16) obtained through PCA and CA in relation to RGB parameters, the estimation of sunflower production, hybrid P64LE25, was analyzed through the analysis of regression.
A general equation in the form of equation (4), was obtained, which facilitated the estimation of sunflower production based on RGB parameters in different combinations (Table 2).

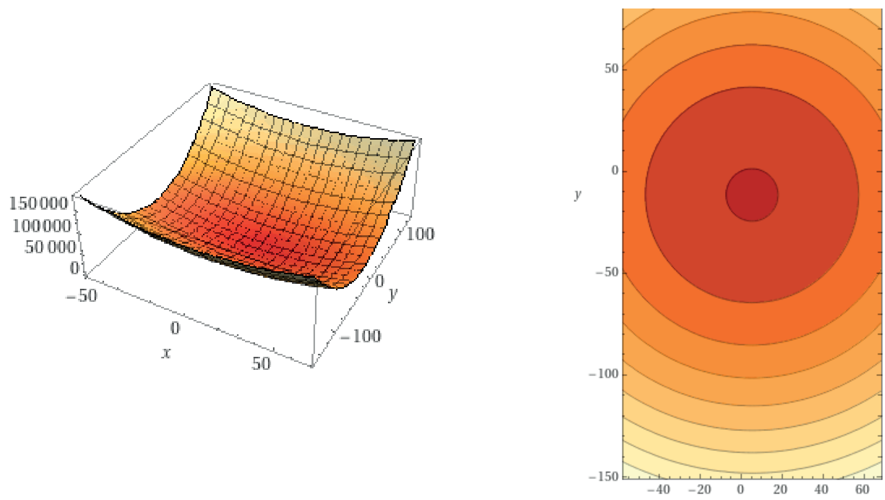
$$Y = ax^2 + by^2 + cx + dy + exy + f \quad (4)$$

where: Y - sunflower production; x and y RGB color parameters in combinations according to Table 2; a, b, c, d, e, f - coefficients of the equation (4) (Table 2).

In relation to R and G, the estimation of Y production (kg ha^{-1}) was made under conditions of $R^2 = 0.999$, $p < 0.001$ (Figure 6). In relation to R and B, production was estimated under conditions of $R^2 = 0.999$, $p < 0.001$, and in relation to G and B, production was estimated under conditions of $R^2 = 0.999$, $p < 0.001$.

Table 2. Values of the coefficients of equation (4) in relation to the combination of RGB color parameters

Coefficients of equation (4)	Values of the coefficients in relation to the combination of RGB parameters		
	x - R, y - G	x - R, y - B	x - G, y - B
a	7.79473007	-0.62392957	-0.99258669
b	7.57721869	-0.65416289	-0.68373757
c	-82.19636475	86.05933728	115.37488965
d	176.42044845	-8.22191157	-59.82367318
e	-15.92116254	0.649730337	1.32391351
f	0	0	0
RMSEP	1.79072	4.58693	3.69784



(a) (b)

Figure 6. The graphic distribution of sunflower production, hybrid P64LE25, depending on the R and G values; (a) 3D model; (b) model in the form of isoquants

Based on the RMSEP parameter, higher accuracy was found in the estimation of production based on R and G parameters (RMSEP = 1.79072), compared to the other combinations of color parameters (RMSEP = 4.58693 in the case of R and B parameters; RMSEP = 3.69784 in the case of parameters G and B), under the conditions of using two color parameters each.

The estimation of sunflower production was considered in relation to all three color parameters, as a direct and interaction effect, and the regression analysis led to equation (5).

$$Y = x^2 + y^2 + z^2 + xy + xz + yz + x + y + z \quad (5)$$

where: x - R color parameter; y - G color parameter; z - B color parameter.

The reliability of the prediction was confirmed by the values $R^2 = 0.999$, $p < 0.001$, RMSEP = 1.46267. The level of statistical safety, assessed on the basis of RMSEP, was higher than the situation in which combinations of two color parameters were used (Table 2).

The graphic distribution of the errors recorded in relation to the image acquisition variants (T1 to T16) and color parameters considered in the regression analysis, are presented in Figure 7.

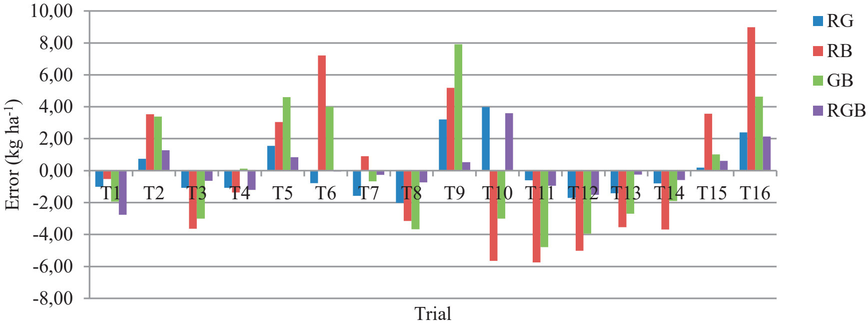


Figure 7. The graphic distribution of the estimation errors of sunflower production, the P64LE25 hybrid, in relation to RGB parameters and the image capture height

Estimating the production of agricultural crops, even during the vegetation period, is important for the appropriate management of the crop, for intervention works, as well as for aspects of harvesting logistics, storage or production utilization (Hassanzadeh et al., 2020; Chang et al., 2023).

In the context of the present study, the prediction of sunflower production was made based on UAV images taken during the fruit development period (BBCH 7 code).

The prediction errors varied in relation to the image capture height, but also in relation to the RGB parameters used in the regression analysis. In the case of using the RGB parameters in combinations of two in the repression analysis, and in relation to the image capture height, the errors reached values of 3.984 kg (RG, at T10), 7.214 kg (RB, at T6) and 7.914 kg (GB, at T9). Under the conditions of using the three RGB parameters together in the regression analysis, the maximum errors only reached 3.592 kg (RGB, at T10). The presentation of the errors in relation to the RGB parameters and the image capture height (T1 to T16) is shown in the dendrogram in Figure 8.

In estimating the biomass production, the N content of the biomass and the yield in sunlight, Vega et al. (2015) communicated that the time of day of image acquisition (UAV), the classification process and the resolution of the images variably influenced the results in study conditions.

The prediction of some physiological indices and production (biomass, seeds, etc.) based on the UAV images was made for different agricultural crops in variable statistical confidence bands, depending on the study conditions. Constantinescu et al. (2018) reported a moderate correlation between chlorophyll and fresh biomass ($r = 0.772$; $p < 0.01$), respectively a strong correlation between chlorophyll and the DGCI index ($r = 0.846$; $p < 0.01$) as part of a study based on UAV images of grass cereals.

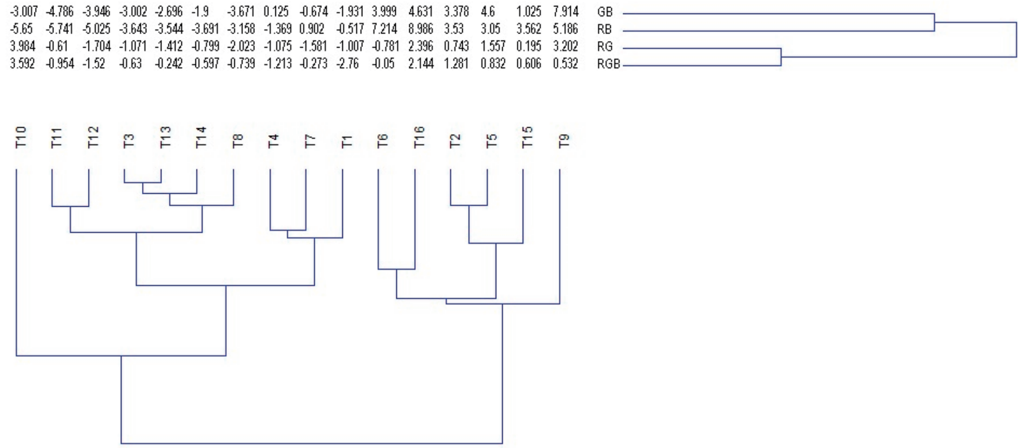


Figure 8. Dendrogram of the distribution of the variants in relation to the error values, results depending on the image capture height and RGB parameters used in the analysis (Coph. corr = 0.839)

In the estimation of biomass, water flow and CO_2 in the sunflower crop through imaging analysis (remote sensing), Pique et al. (2020), obtained models that facilitated the estimation of the considered parameters under high statistical safety conditions ($R^2 = 0.79$ to $R^2 = 0.83$).

In the conditions of the study of the sunflower weed spectrum based on aerial images (UAV) at two flight heights (30 and 60 m altitude), López-Granados et al. (2016), found that high accuracy (~100%) in the discrimination of weeds was recorded under the conditions of using the multispectral camera, regardless of the flight height, and low accuracy (50-60%) was obtained under the conditions of using the camera in the spectrum visible.

In estimating the lodging phenomenon in sunflowers based on UAV images, Li et al. (2021) obtained 12 models and found better accuracy in the assessment/characterization of the studied phenomenon (lodging) by using NIR information to RGB ones, than by using RGB alone. Studying the same sunflower phenomenon (lodging) based on UAV imagery, but with different analysis methods, Song et al. (2020) found that by using improved methods, the level of estimation was more accurate.

The results communicated through this study are comparable to similar studies on sunflower, but also on other agricultural crops, and can contribute to the improvement of agricultural technologies and an adequate management of crops.

CONCLUSIONS

The images taken with the drone, in the height range between 3 m and 50 m, captured the sunflower crop status, the P64LE25 hybrid, and the image analysis facilitated obtaining the values for the RGB color parameters in conditions of statistical safety.

Based on the recorded values, it was possible to characterize the sunflower crop, and different levels of interdependence between the RGB values, described by polynomial or linear equations, were identified.

The distribution and grouping based on the affinity of the variants was possible through PCA and cluster analysis, based on the RGB values and the image acquisition heights (T1, to T16).

The estimation of sunflower production, based on the RGB parameters obtained from the aerial images (UAV) taken in stage 7 BBCH, was possible under statistical safety conditions. Variable accuracy of production estimation was recorded, in relation to the color parameters used (RGB) and the image capture height.

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Pyrenophora teres, HOST-PATHOGEN INTERACTION IN BARLEY UNDER SOME SEED TREATMENT CONDITIONS

Alin CARABET[1], Ioana GROZEA[1], Dan MANEA[1], Otilia COTUNA[1], Daniela SCEDEI[1], Maria IAMANDEI[2], Ramona STEF[1]

[1]University of Life Sciences “King Mihai I” from Timisoara, 119 Aradului Blvd, Timisoara, Romania
[2]Research - Development Institute for Plant Protection Bucharest, Romania

Corresponding author email: chirita_ramona@yahoo.com

Abstract

The paper aimed to present alternative against the pathogen Pyrenophora teres (anamorph Drechslera teres) which parasitizes barley crop in western parts of Romania even beginning with late autumn period, observing the evolution and symptoms spreading of this fungus. Across the world, Pyrenophora teres causing the net blotch of barley is regarded as the major foliar disease in Hordeum vulgare L. Throughout its two form of Phyrenophora teres, however different from genetical point of view, P. teres f. sp. maculata and P. teres f. sp. teres causes economic losses by reducing yield and grain quality and poor emergence in area with a high biological pressure of the pathogen. The trial extends for two years (2019-2020, 2020-2021) in the same area of cropping (monoculture system) using same seed treatment list and following the seed-borne cereal fungi assessment protocol [(EPPO 1/19 (4)]. Trial setup consisted in complete randomized blocks, 6 treatments like fludioxinil, fluxapyroxad and mixture, every plot measuring 10 m^2 and observations performed an al plants/1 m in length sample. When treated with two active ingredients the result obtained exerts an efficacy up to 98%, maintained in both trial years compared with the untreated plots where the pathogen was well established.

***Key words**: Pyrenophora teres, net blotch, pathogen, barley, efficacy.*

INTRODUCTION

Net form net blotch (NFNB) of barley (*Hordeum vulgare*) is caused by the fungal pathogen *Pyrenophora teres* f. *teres*.

Globally, NFNB results in regular yield losses of between 10 and 40% with the potential for complete losses in environmental settings favourable to the pathogen, namely, susceptible cultivars with high sustained humidity and the absence of fungicides (Mathre et al., 1997; Liu et al., 2011).

Reduced or no-till agricultural practices have most probably contributed to the increase in importance of both forms net and spot form net blotch (NFNB and SFNB) disease (Mathre, 1997; McLean et al., 2009; Shipton et al., 1973); however, the susceptibility of current cultivars and trends in environmental conditions cannot be ruled out as contributing factors to the increased importance of the disease.

Originally named *Helminthosporium teres* (Sacc.) in 1809, the fungus was renamed *P. teres* Drechs. (anamorph *Drechslera teres* (Sacc.) Shoem.) in 1930 (Shoemaker, 1959).

P. teres was subsequently divided into the two forms *P. teres* f. *teres* and *P. teres* f. *maculata* by Smedegård-Petersen (1971) based on the lesion types. *P. teres* f. *teres* develops necrotic lesions with distinct striations, developing the net-like pattern for which it was named. *P. teres* f. *maculata* develops oval necrotic lesions with a chlorotic halo (Shipton et al., 1973; Smedegård-Petersen, 1971).

Both forms induce a combination of brown necrotic spots/lesions and general chlorosis in affected barley leaves. The brown necrotic spots/lesions are induced by proteinaceous toxins (Sarpeleh et al., 2008; Bach et al., 1979 quoted by Sarpeleh et al., 2008) while the chlorosis has been shown to be induced by low molecular weight compounds (LMWCs) isolated from culture filtrates of *P. teres* (Weiergang et al., 2002).

The pathogen can infect and cause disease on leaves, leaf sheaths, stems and kernels of barley plants. Infection of the kernel can transfer the pathogen into a new field and can serve as primary inoculum (Liu et al., 2011). Crop rotation, avoiding barley monoculture and eliminating or reducing primary inoculum in the field are means preventing the pathogen's development (Liu et al., 2011; Vasilieva et al., 2022).

Besides avoiding monoculture and use of seed treatments a chois of biocontrol methods, against diseases and pests, can lead to a safer crop production (Röhner et al., 2004; Grozea et al., 2015; Virteiu et al., 2016)

Figure 2 Trial setup and randomization

Figure 3. The research field in spring

MATERIALS AND METHODS

To characterize the interaction between pathogen *Pyrenophora teres* with the host plant we used inoculated barley seeds (GEVES - France) and coated with different active ingredients, drilled in the last decade of November in year 2019, respectively 2020 in western part of Romania.

We chose various active substances as seed treatments: fluxapyroxad (100 ml/100 kg), fludioxonil (1.5 l/t), sedaxan (2 l/t), mixture of protioconazole and tebuconazole (0.5 l/t) and sedaxan mixed with fludioxonil in a rate of 2 l/t.

The trial set up was done following EPPO and CEB guidelines for seed borne cereal fungi and seed treatments, EPPO 1/19 (4) Seed borne cereal fungi; 1/135 (4) Phytotoxicity assessment; 1/181 (4) Conduct and reporting of efficacy evaluation of trials including good experimental practices, CEB M042 Seed treatment.

Placed as randomised complete block, the plot has 10 sqm (1 m wide and 10 m length) 8 rows each plot (Figures 1-3).

Figure 1. Plots drilling with inoculated seeds

We followed general phytotoxicity, crop vigour at early crop emergence and fully emerged (BBCH 12-14), speed of emergence and number of plant emerged, disease control (diseased plant/plot) assessing 10 samples/plot consisting in 30 plants per sample (Figures 4-6).

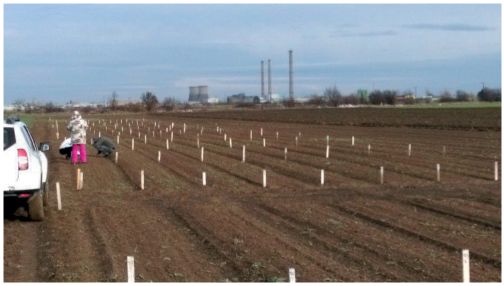

Figure 4. Performing assessment in late 2019

Figure 5. Assessing number of plants emerged and speed emergence

Figure 6. Assessment of diseased plant per plot

The data collected were statistically displayed throughout ANOVA and Student-Newman Keuls mean comparison test.

RESULTS AND DISCUSSIONS

Following the study protocol, general plant phytotoxicity and crop vigour at the first plots fully emerged were assessed. In tyhe plots where seeds treated with sedaxan mixed with fludioxonil and protioconazole and tebuconazole as well the emergence and plant vigour was at highest level of 100%. The lowest emergence rate was recorded (in both experimental years 2019-2020) in untreated inoculated variant where the emergence noted was 70% as a mean value followed by untreated and not inoculated with 85%. The plant vigour proved to be in a strong relationship with the plant emergence rate, the most vigorous plants were those in the treatment 3 and 7, sedaxan/fludioxinil and protioconazole/ tebuconazole respectively (Table 1, Figure 7).

Table 1. Plant vigour at BBCH 11

	Untreated		Untreated inoculated		Sedaxan + Fludioxonil		Fluxapyroxad		Sedaxan		Fludioxonil		Propiconazole + Tebuconazole	
	% general phyto	% vigor of plants	% general phyto	% vigor of plants	% general phyto	% vigor of plants	% general phyto	% vigor of plants	% general phyto	% vigor of plants	% general phyto	% vigor of plants	% general phyto	% vigor of plants
	0	90	0	70	0	100	0	100	0	90	0	100	0	100
	0	90	0	70	0	100	0	90	0	100	0	90	0	100
	0	80	0	70	0	100	0	90	0	100	0	100	0	100
	0	90	0	70	0	100	0	100	0	90	0	90	0	100
Average		87.5		70		100		95		95		95		100

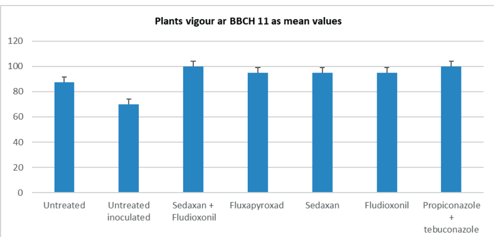

Figure 7. Plant vigour at fully emergence, BBCH 11

Following seeds treatments with the test items no general phytotoxicity was observed.

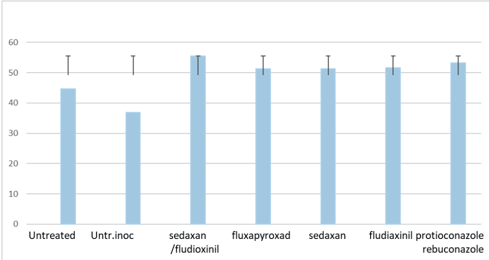

Figure 8. Plant emergence (plant/m^2), 2019 St. dev. at $p < 0.05$

In respect of emerged plants/sqm, in year 2019, the differences are notable looking forward to yield potential being corelated to number of ears per m^2. It can be observed that in the plots where drilled untreated and untreated inoculated seeds, the number of plants was low, 36.8 plants per square meter in untreated inoculated plots respectively 44.7 plants/m^2 in untreated plots. The highest plant number was exerted in the plots where seeds are treated with sedaxan mixed with fludioxinil and protioconazole plus tebuconazole, 55.5 plants/m^2 respectively 53.2 plants/m^2 (Figure 8).

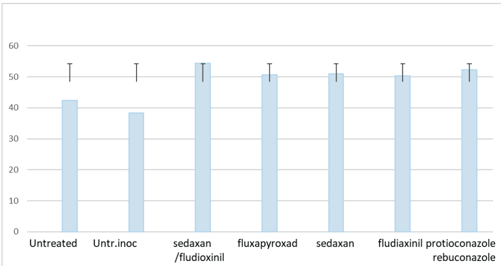

Figure 9. Plant emergence (plant/m^2), 2020 St. dev. at $p < 0.05$

In experimental year 2020, the plants number per square meter did not change dramatically, dough a slightly increase was observed in the plots where untreated inoculated seed were drilled, in this case the emergence was 38.8 plants/m^2 (Figure 9). The highest emergence rate was recorded in variants where the seeds were coated with sedaxan plus fludioxinil at a rate of 2 l/t, with 54.3 plants/m^2. The variants where protioconazole and tebuconazole mixture were applied the results were pretty much in the same range as experimental year 2019, meaning 52.2 plants/m^2.

Table 2. Number of plants/m^2 in relation with untreated inoculated variant

	Untreated inoculated	Untreated	Fluxapyroxad	Fludioxonil	Sedaxan	Propiconazole + Tebuconazole	Sedaxan + Fludioxonil
Mean	36.81	44.71	55.52	51.24	51.29	51.57	53.24
St.dev	6.194	11.037	8.091	8.348	7.370	7.580	7.155
in % to UC	100	121	151	139	139	140	145

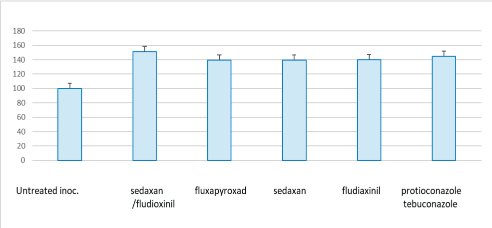

Figure 10. Plants per sqm in related to untreated inoculated

Regarding the disease control/diseased plants per variant, in year 2019 significant differences were found at p<0.05 (Figure 11).

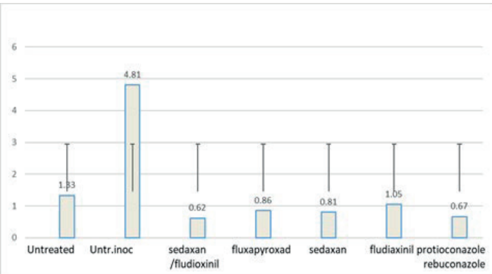

Figure 11. Diseased plants, 2019 significant difference at p < 0.05

The highest number of diseased plants, showing symptoms, was observed in variant where untreated inoculated seeds were drilled, namely 4.8 plants as mean value per variant. The lowest number of infected plants was achieved in variants where active ingredients sedaxan and fludioxinil was used to control seedborne pathogen *Pyrenophora teres* with a mean value of 0.62 plants infected. Despite the fact that untreated variants were drilled in conditions of natural occurring infection, the diseased plant rate lays in the range of 1.33 infected plants compared to 1.05 in the case of fludioxinil treatment (Figure 12)

In the next experimental year, 2020, however, like in previous year, significant differences were recorded between treatments.

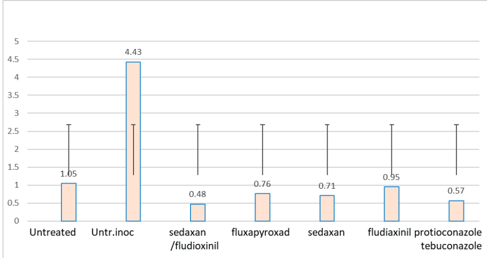

Figure 12. Diseased plants, 2020 significant difference at p < 0.05

Comparing untreated inoculated (4.43 diseased plants) variant with sedaxan and fludioxinil mixture one (0.48 diseased plants) and as Figure 10 shows, signifficant differences can be observed. The variants treated with protioconazole and tebuconazole. The untreated variant recorded an average of 1.05 diseased plants.

Treatments 4 and 5, namely seeds treated with fluxapyroxad at a rate of 100 ml/100 kg respectively treated with sedaxan at a rate of 2 l/t exerted no significant differences, the diseased plants number recorded was 0.76 and 0.71. whilst in experimental 2019 the infected plants as a mean value laid in the range of 0.81 in the case of sedaxan and 0.86 in the case of fluxapyroxad treatment.

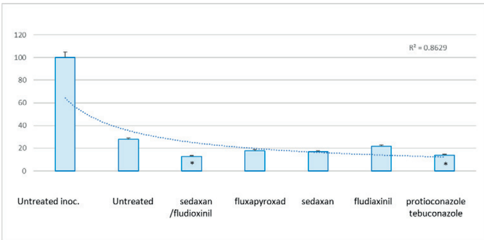

Figure 13. Effectiveness of seed treatments in year 2020, significant difference at p<0.05

The efficacy achieved, in year 2020 (Figure 13), significant differences in the plots treated with sedaxan and fludioxinil mixture, where 13% of the exanimated plants compared with untreated inoculated variant, meaning an 87% of control. However, the variant where mixture of protioconazole plus tebuconazole was used the results were pretty much in line with the one obtained in above mentioned mixture (sedaxan and fludioxinil) with a efficacy up to 86% compared with untreated inoculated variant.

In respect of disease control, net blotch, can be approached trough various methods, seed treatment playing an important role in disease epidemiology as an interrupting factor reducing the potential for secondary infections in the throughout vegetation period.

Although, the need of healthy food orientated to a healthy human diet and production of safe animal proteins (Manea et al., 2021), using the seed treatment with a right active substance which provide an extended period in control of soilborne pathogens can be linked with a sustainable, less costly and safer crop production at a farm level.

CONCLUSIONS

Pyrenophora teres causing net blotch is considered to be one of the most devastating diseases of cultivated barley (Weiland et al., 1999). Therefore, new technologies and active substances are developed including the ones used in seeds treatments.

The trial extends for two years (2019-2020, 2020-2021) in the same area of cropping (monoculture system) using same seed treatment list and following the seed-borne cereal fungi assessment protocol.

The lowest emergence rate was recorded (in both experimental years 2019-2020) in untreated inoculated variant where the emergence noted was 70% as a mean value followed by untreated and not inoculated with 85%, caused either by compromised germination trough presence of seedborne pathogen in inoculated seeds or by biological soil reserve.

Nevertheless, in the variants treated with mixtures sedaxan and fludioxinil respectively protioconazole and tebuconazole the emergence rate registered was 100%

Significant differences in respect of diseased plants per plots as a mean value was exerted by mixture sedaxan and fludioxinil with average of 0.48 diseased plants compared with untreated inoculated with an average of 4.43 – 4.81 diseased plants.

Fluxapyroxad used at a rate of 100 ml/100 kg and sedaxan used at a rate of 2 l/t exerted no significant differences, the diseased plants number recorded was 0.76 and 0.71.

In lasts years the farmers began to pay more attention to new technologies implemented in whole food chain, especially entire technological process related to grain crops production where barley is included. So, there is a need to implement all the necessary steps to obtain productive and safer crops.

Other than usual procedures used in agriculture, such as soil preparation, crop rotation, and in vegetation treatments against pest and diseases, treatments, the use of treated seeds plays an key role in order to achieve profitable agricultural crops throughout decreasing of biological pressure of net blotch causing pathogen, *Pyrenophora teres,* as a soil seed borne pathogen, diminishing the second infection wave during the vegetation period of the crop trough control within the crop and on secondary host as well.

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CONTRIBUTIONS TO THE ONTOGENETIC STUDY ON THE SPECIES *Silphium perfoliatum* L. UNDER THE CONDITIONS OF THE REPUBLIC OF MOLDOVA

Natalia CÎRLIG

"Alexandru Ciubotaru" National Botanical Garden (Institute), 18 Padurii Street, MD 2002, Chisinau, Republic of Moldova

Corresponding author email: nataliacirlig86@gmail.com

Abstract

The ontogenetic peculiarities of the species Silphium perfoliatum L. cv. 'Vital', known as a high-potential melliferous, fodder and energy species, the periods and age stages that the plants go through under the climatic conditions of the Republic of Moldova have been described. The research was carried out in the experimental sectors of the "Alexandru Ciubotaru" National Botanical Garden (Institute), during four growing seasons. In the ontogenetic cycle, 4 ontogenetic periods (latent, pregenerative, generative, postgenerative) and 6 age stages (seed, seedling, juvenile, immature, virginal, senile) were described. The end of the ontogenesis in S. perfoliatum, in the Botanical Garden, has not been recorded yet. The duration of the active growth period varies between 197 and 234 days depending on the weather conditions recorded in the Republic of Moldova and the age of the plants. S. perfoliatum plants are characterized by long flowering stage, being able to provide honeybees and other pollinating insects with pollen and nectar at the end of summer - beginning of autumn.

***Key words**: Silphium perfoliatum, ontogenetic periods, age stages, phenological stages.*

INTRODUCTION

Silphium perfoliatum L. is a species in the family Asteraceae Bercht. & J. Presl, tribe Heliantheae, genus *Silphium;* it is native to North America. The species of this genus are diploid, with seven pairs of chromosomes, and genome size 2.5 times larger than in sunflower (2C = 16.6-16.9 pg) (Bai et al., 2012), which allowed the researchers to demonstrate the polymorphism of the species. In some older sources (Hegi, 1906; Poletico & Mishenkova, 1967) cup plant has been described using several synonyms: *S. connatum* L.; *S. scabrum* Moench.; *S. conjuctum* Willag.; *S. integrifolium* Michx.; *S. laeve* Hook.; *S. speciosum* Rybd. Currently, according to The Plant List, the accepted name of this species is *Silphium perfoliatum* L.

It is a mycorrhizal species (Dhillion & Friese 1992), with strong roots, which grow deep into the soil (Wynia, 2009), is drought tolerant (Ouyang et al. 2007) and makes good use of wet and eroded soils, contaminated with heavy metals, but does not tolerate well marshy soils (Țîței & Roșca, 2021). Recently, cup plant was included in the list of eligible species for areas of ecological interest, being recognized by the European Union for the benefits brought to the environment and the soil (European Commission, 2018). In Europe, *S. perfoliatum* was introduced in the 18th century, being initially used as an ornamental plant in gardens and parks in Germany, France, Switzerland and the Great Britain (Vavilov & Condratiev, 1975; Uteus, 1991). Currently, it is known as a non-traditional perennial, herbaceous forage crop that produces large amounts of fresh mass with rich biochemical content. It is resistant to unfavourable conditions (-30…+30°C) and, in spring, comes out of dormancy when the soil temperature reaches values of 3-5°C. Cup plant is a mesophile and prefers loamy-clayey and deeply loosened clayey soils (Țîței & Roșca, 2021). In "Al. Ciubotaru" (National) Botanical Garden (NBGI), the species *S. perfoliatum* was introduced in the second half of the 20th century; from the initial plants, biological material was later selected for breeding. Researchers from NBGI, as a result of many experiences and analyses, created a new, indigenous cultivar of *S. perfoliatum* - 'Vigor', registered in the Catalogue of Plant Varieties of the Republic of Moldova in 2012 and patented at the State Agency for Intellectual Property

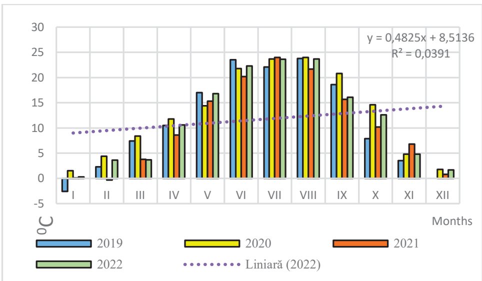
(AGEPI) in 2016. The new plants obtained are characterized by a greater amount of green mass, increased resistance to adverse conditions and high protein content. The basic qualities of cup plant are high productivity and viability of about 15 years on the same plot. As a fodder crop, it is eaten by a wide range of animals (cattle, sheep, pigs, goats, rabbits etc.), the green mass being used as fodder and for the preparation of vitamin-fortified flour. The productivity of plants is 100 (120) t/ha (Abramov, 1992; Țîței & Roșca, 2021). *S. perfoliatum* is also known as a high-potential honey plant (150-450-560 kg honey/ha) (Koltowski, 2005), the nectar productivity can vary, between 205.2 and 611.6 kg/ha, being closely related to the quality of the soil and the amount of mineral fertilizers applied (Savin & Gudimova, 2019). In addition to nectar, cup plant is also a source of bee bread (flower pollen used by bees as food for the larvae in the hive), besides, cup plant honey does not crystallize for a long time, being a source of food for bees during the winter (Abramov 1992). In the flowering stage, the flowers are visited by a large spectrum of pollinating and honey-producing insects (Cîrlig, 2022); as a result, seed productivity also increases.
Ontogenesis is the sequence of all the developmental stages of an individual from the embryo stage to the death of the individual or to the death of all vegetative descendants (Smelov, 1937), a process that does not take place permanently in the same way, and it can be different under various environmental conditions but also under similar conditions. The main processes of ontogenesis are: growth, development, aging (devitalisation) and rejuvenation (Timciuc et al., 2019). Smirnova et al. (2002), explain that determining the absolute age of plants is sometimes impossible due to the permanent renewal of perennial organs, but the classification of the ontogenetic state of plants is quite real.
The researches initiated, carried out and described in this article are aimed at highlighting the ontogenetic peculiarities of *S. perfoliatum* plants under the climatic conditions of the Republic of Moldova, determining the life periods and age stages characteristic of this species in correlation with the meteorological conditions recorded in different years.

MATERIALS AND METHODS

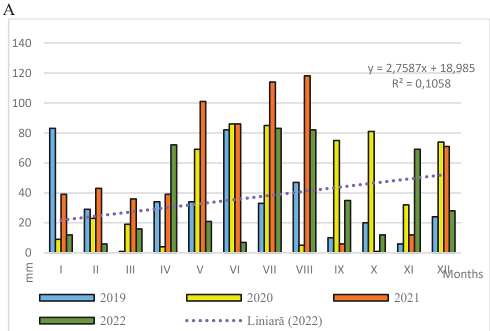
The plants of the species *Silphium perfoliatum* L., cultivar 'Vigor', served as subjects for the study. The experiments were conducted in the experimental sector of the “Plant Resources” Laboratory of "Alexandru Ciubotaru" (National) Botanical Garden. NGBI is found in the South-East part of Chisinau, on an area of 104 ha. NBGI hosts a unique gene pool of plants, its collections count about 8500 taxa of spontaneous, fodder, energy, honey, medicinal, aromatic, spicy and ornamental plants.
The research was carried out in four growing seasons (2019, 2020, 2021, 2022) being studied plants aged 1, 2 and 10 years, in order to establish the full ontogenetic cycle.
The ontogenetic study was conducted according to the methodology proposed by Rabotnov (1950) who proposed the principle of periodization of ontogenesis, later completed by Uranov (1975) and by Florea (2006). Phenological observations were made and plant growth and development stages were evaluated according to the methodological guidelines used in the Botanical Garden (Metodica fenologhiceskih nabliudenii v botanicheskih sadah SSSR 1972). Seed germination was researched according to the indications mentioned in the book “Metodicheskie ucazania po semenovedeniu introdutentov” (1980). Special attention was paid to the flowering stage of the plants, being researched as plants with melliferous potential. The planting material was obtained by applying the seedling production methodology used in the cultivation of vegetables (Taracanov, 2003). The experiments were mounted in cell trays in protected environment, followed by the appropriate stages of obtaining healthy seedlings.
The climatic conditions were systematized and characterized with the help of the information provided by the State Hydrometeorological Service, comparatively, in the years during which the research was carried out.

RESULTS AND DISCUSSIONS

The climatic conditions of the Republic of Moldova (Figure 1 A, B) with inhomogeneous temperature regime and amount of precipitation, are favourable for the growth and development of cup plant and the unfolding of its ontogenetic program. The agro-meteorological conditions recorded during the growing season of 2022, characterized by high temperatures and precipitation deficit (spring with fluctuating temperatures, hot summer) did not affect the growth and development of plants, thus, they managed to complete the full cycle of vegetative and generative phenological stages. Besides, the favourable temperatures in autumn favoured the prolongation of the flowering stage in some specimens, the plants becoming an additional source of food for entomofauna. As compared with 2021, the air temperature was by 1.0-1.5°C higher and the amount of precipitation was lower, and compared with 2020, the atmospheric temperature was by 0.5-1.5°C lower and the amount of precipitation - higher.



A



B

Figure 1. Main meteorological indices in the 2019-2022 research periods: A - average monthly temperature; B - the monthly amount of atmospheric precipitation

The variations in temperature and precipitation did not significantly influence the annual development of plants, only some changes occurred in the seasonal growth rate and duration of the growth season. Regardless of the weather conditions recorded in Republic of Moldova, *S. perfoliatum* plants, starting from the 2nd-3rd years of development, went through the ontogenetic cycle, producing viable seeds by the end of the growing season.

The research of ontogenetic features is an important step in studying the growth and development of plants, their dependence on climatic conditions and the role of plants in their respective biocenosis. The vital form of *S. perfoliatum*, under the climatic conditions of the Republic of Moldova, is perennial, polycarpic herbaceous plant. Some authors describe the ontogenetic cycle of cup plant as a series of development cycles of monocarpic shoots that grow and die annually. The lifespan of each annual shoot is about 8-10 months (Vavilov & Condratiev, 1975).

The research on the biological cycle of *S. perfoliatum* plants, under the climatic conditions of the Republic of Moldova, allowed determining the life periods and age stages characteristic of the species. Four periods (latent, pregenerative, generative (g), postgenerative) and 4 stages (seeds - sm, seedling (plantlet) - pl; juvenile - j; immature - im; virginal - v; senile - s) were described. The biological cycle of cup plant is divided into the active growth period and the dormancy period. The duration of the active growth period is 197-234 days. *S. perfoliatum* plants are characterized by: high rate of growth and development, intensive growth process, high productivity of seeds.

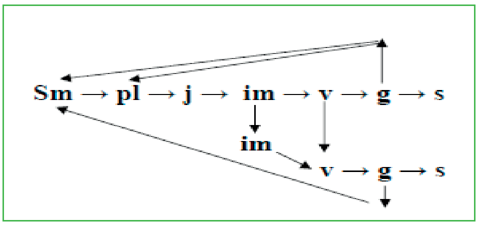


Figure 2. The scheme of the ontogenetic program of *S. perfoliatum* plants under the conditions of the Republic of Moldova

The research carried out demonstrated that the *S. perfoliatum* individual plants from the territory of the "Alexandru Ciubotaru" National Botanical Garden (Institute), depending on the pace of development, can adopt several variants of the ontogenetic program (Figure 2), and the climatic conditions are favourable for the fulfilment of the biological potential of the species.

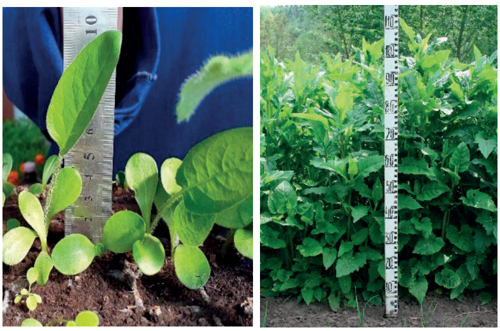
I. The latent period includes the time from the fertilization of the ovule to the germination of the seeds.

The seed stage. Cup plant produces seeds every growing season. The seed material sown under laboratory conditions, in cell trays, at a depth of 0.8-1.0 cm in soil, being provided favourable conditions for development, began to germinate (Figure 3) at the optimal temperature of 12-15°C. The weight of 1000 achenes is 22-24 g, and the seed productivity reaches 290-450 kg/ha (Țîței & Roșca, 2021).

Figure 3. The stages of seed germination in *S. perfoliatum*

Once the seeds germinate, a new ontogenetic cycle of plant development is initiated.

II. Pregenerative period. It includes the seedling, juvenile, immature and virginal age stages. The growth and development of plants in these stages are closely connected to the weather conditions, thus, unfavourable conditions can make these stages last longer.



A B

Figure 4. *S. perfoliatum*: A - seedling stage; B - virginal stage

The seedling stage. *S. perfoliatum* plants at this stage were researched under indoor conditions, namely, planting material obtained in cell trays, at a temperature of 18-20°C. According to the position of the cotyledons in the germination process, cup plant is characterized by epigeal germination. Initially, radicles with absorbent hairs develop. Then, after 5-6 days from the time of incorporation, the thickened, white-greenish hypocotyl emerges at the soil surface. In the next 2 days, the cotyledons come out entirely at the soil surface, being 1.5-1.8 cm long, pale green or yellowish. It takes 14-18 days from the time the seeds are incorporated into the soil until the appearance of the first true leaf (Figure 4A). The plants are in the state of seedling with cotyledons and a true leaf for about 12-18 days. After the 35-39th day, the second true leaf develops.

The juvenile stage. It is characterized by the more intense development of the first 2-4 true leaves. The leaves are petiolate, pubescent, 8-12 cm long and 3.5-5.0 cm wide. At this stage, plants depend highly on temperature and humidity. At the end of this stage, from the buds in the axils of the cotyledons, other true leaves begin developing. The root system expands, providing the plants with a more stable connection to the soil.

S. perfoliatum can be propagated by seeds directly in open ground as well as by transplanting seedlings obtained indoors. The production of seedlings for transplantation seems to be more expensive and a lengthy process, but some researchers describe this method as more efficient, as a result, the plantation is established earlier, is more uniform, thus making use of the selected land more efficiently and the yield are higher; the explanation would be the low germination capacity of seeds in open ground (Gansberger et al., 2015; Ruf et al., 2019).

Gansberger et al. (2015), Pan et al. (2011), Schoo et al. (2017) recommended the optimal planting density of 40,000 plants/ha, that is, about 4 plants/m^2.

The immature stage. At this stage, the root system develops more intensively and branches. The aerial part of the plants is a rosette of 7-14 leaves, the growth rate of the foliage is slower, the plants in open ground reach a maximum height of 32-45 cm and remain so until the end of the growing season, in late autumn. In the first year of vegetation, cup plant goes through the ontogenetic cycle until the immature stage of development, which coincides with the phenological stage – leaf development. During this period it is not recommended to cut the plants for green mass, because the resources of the plants are focused on the formation of the root system and the leaf rosette.

In the second year of life, *S. perfoliatum* plants come out of dormancy early, when the atmospheric temperature reaches 3-5°C. They are characterized by rapid growth, with intensive formation of the leaf rosette and stems in a short time, as well as morphological features similar to the mature plant, which already correspond to **the virginal stage** (Figure 4B). The shape of the leaves is similar to that in the immature stage. The underground part is strongly developed and the plants are prepared for the next important period in their life cycle.

In the second year of life, in the spring season, in open field, *S. perfoliatum* plants reach the virginal stage. In 30-35 days from the start of the growing season, the plants reach a height of 18-25 cm and form a rosette of 6-10 leaves. In the next phase, the green mass is formed more rapidly, and the stems grow very fast (h: 250-370 cm). The stems are 4-angled, erect, with hairs. Plants can produce a different number of flower stalks (7-15-23 depending on the age of the plants). The leaves grow larger, are light green, heart-shaped, rough, opposite. The lower leaves are petiolate, but those growing in the middle and apical part of the stem are fused around the stem, forming a cup, which helps the plant use moisture more efficiently. The leaves growing at the base of the stem (also called "spring" leaves) turn brown when the plants enter the flowering phase. The leaves that are developed during the summer period are more resistant and turn brown only in autumn when temperatures below 0°C are recorded, at the end of the growing season. Mature leaves are 25-35 cm long and 16-22 cm wide (Țîței et al., 2020). The root system - a well-developed tap root - has high capacity for branching and can grow about 3.5 m deep in the soil (Ţîţei & Roşca, 2021.).

Starting from the summer of the second year of life (sometimes from the third year, depending on the recorded weather conditions and the time of planting in the field), the plants go into the generative period.

III. The generative period. In this period, flower stalks and flower buds develop. The assimilation surface reaches maximum size. During a growing season, *S. perfoliatum* plants go through the entire cycle of vegetative and generative phenological stages - bloom, produce fruits and viable seeds. In the generative phase (Figure 5A), the growth of the plant and the formation of the plant mass slows down. The generative phenological stages are staggered. The flowering stage extends over a period of 51-63 days and occurs at the end of June - August or in July - September, depending on the weather conditions (Table 1).

Table 1. The life cycle of the species *S. perfoliatum* throughout a growing season

No.	Life period	Age stage	Length (days)	Phenological stage
I	Latent	Seed (sm)		Morphological dormancy
II	Pregnerative	Seedling (pl)	<30	Germination
		Juvenile (j)	18-75	The development of the first true leaves and of the root system
		Immature (im)		
		Virginal (v)	25-36	Full development of foliage and root system
III	Generative	Generative (g)	115-130	Budding
				Flowering
				Fruit development
				Seed ripening
IV	Postgenerative	Senile (s)	10-15	The end of the growing season
The length of the growing season			197-234 days	

In some specimens, the flowering stage lasts even longer, having solitary flowers until the middle of October. During this period, solitary specimens of insects can be noticed on the flowers. An inflorescence consists of 20-30 yellow flowers of 3-5 cm in diameter, each flower produces by 20-30 seeds.

Cup plant flowers are attractive to a wide range of honeybees and pollinators, providing pollen and nectar until autumn, when food sources are scarce. As a result of the entomological monitoring carried out at the National Botanical Garden (Institute) "A. Ciubotaru", 10 species of honey-producing and pollinating insects visiting cup plant flowers, belonging to 6 families and 4 orders, were identified. The species of the Apidae family (*Apis mellifera, Bombus terestris, B. lapidarius*) had the maximum frequency on the flowers (Cîrlig, 2022). The research carried out by Wroblewska (1997) demonstrated the role of insects (especially the honey bee) on the number of seeds produced as a result of pollination. In the inflorescences isolated from insects, only 5-10% of seeds were produced, and in those pollinated by bees, 70-80% of seeds were formed. Cup plant is considered a high-potential honey plant in England, Germany, Russia, Bashkortostan, Bulgaria (Daniel, 1984; Wroblewska, 1997; Hoves, 2017), being able to provide a late-season source of nectar and pollen for insects. An inflorescence of *S. perfoliatum* produces, on average, 122 disk florets, and a disk contains about 14200 pollen grains (Mueller et al., 2020).

Mueller et al. (2020) calculated the average productivity of a cup plant flower: 1.75 x 10^6 pollen grains, 12.5 x 10^{12} pollen grains/ha 80 kg/ha nectar sugar each season, which would provide the necessary food for 34 honey bee larvae per season and 6 worker honey bee per day.

A B

Figure 5. *S. perfoliatum*: A - generative phase, flowering stage; B - senescence stage

The post-generative period in higher plants is characterized by the disintegration of the root system and the death of the whole organism. The state of *S. perfoliatum* plants at the end of the growing season corresponds to the **senescence stage** (Figure 5B), the stems turn brown, dehydrate, generative shoots are no longer produced, the area with dead tissues predominates. However, these physiological processes do not mean the end of ontogenesis of the species. Under the climatic conditions of the Republic of Moldova, *S. perfoliatum* plants have not reached this stage yet.

Once the growing season ends, the plants are of interest for biofuel production. The biomethane production potential is 230 l/kg and the gross calorific value is 17.8 J g^{-1} (Țîței et al., 2020).

CONCLUSIONS

The research carried out at the "Alexandru Ciubotaru" National Botanical Garden (Institute) on *Silphium perfoliatum* L. plants demonstrates the high adaptability of plants to the climatic conditions of the Republic of Moldova. During a growing season, plants go through the ontogenetic cycle of development which includes 4 life periods: latent, pregenerative, generative, postgenerative and six age stages: seed, seedling, immature, juvenile, virginal and senile. In the seedling and immature stages, plant development highly depends on climatic conditions. The temperatures below 0°C in spring can slow down the growth of vegetative organs for a few days. In the first year of life, cup plant goes through the ontogenetic cycle until the immature stage of development, the root system and the leaf rosette develop intensively. The following growing season, the plants go into the virginal stage, during which the plants have a high rate of growth and development of vigorous stems and foliage. The generative period is long and coincides with the generative phenological stages characteristic of this species: budding, flowering, fruit development and seed ripening. The flowering stage lasts about 51-60 days, depending on the recorded weather conditions, becoming a source of food for honeybees and pollinating insects for a long period, and lasts till autumn when very few other species are blooming. The analysis of specialized literature demonstrates the interest of researchers towards *S. perfoliatum* - a multi-purpose crop, characterized by high adaptability and resistance to various climatic conditions, fast growth and high melliferous potential.

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INFLUENCE OF SATURATION OF SHORT-TERM CROP ROTATIONS WITH SUNFLOWER ON SOME AGROPHYSICAL PARAMETERS OF TYPICAL CHERNOZEM

Zinaida DEHTIAROVA, Sergii KUDRIA, Yurii DEHTIAROV, Nadiia KUDRIA

State Biotechnological University, p/o Dokuchaevske, Kharkiv, Ukraine

Corresponding author email: zinaidasamosvat@gmail.com

Abstract

The article presents the results of the 2020-2021 research carried out in the experimental field of Kharkiv National Agrarian University named after V. V. Dokuchaev, located in the area of the Left Bank Forest-Steppe of Ukraine. The experiment was conducted on typical heavy loamy chernozem on loess-like loam. We aimed to find out the possibility of scientifically based expansion of sunflower crops and determine its productivity in field crop rotations. The effects of increasing the share of sunflower in short-term crop rotations on some agrophysical parameters in the topsoil (0-30 cm) were determined. The results show the influence of crop rotations with different sunflower saturation on the structure-aggregate composition, soil density, etc. Correlation analysis between these indicators and sunflower yield was also carried out. During the period of research, it was found that the increase in the share of sunflower in short-term crop rotations did not lead to a significant deterioration in the fertility of typical chernozem.

Key words*: sunflower, saturation, agrophysical parameters, typical chernozem.*

INTRODUCTION

Sunflower is one of the most widely grown crops in Ukraine. This is due to the high profitability of its production, which in 2021-2022 amounted to 61%, while grain production was only 30-35%. Low labor costs for cultivation, an unlimited domestic market, and sales of products on the world market make sunflower profitable for cultivation (Debaeke, et al., 2017). All soils have their own agrophysical and agrochemical properties, which depend on the nature of the mineral and organic components, their relative amounts, and the ways in which minerals and organic matter interact (Kharytonov et al., 2019). Soil structure, density, water, air, heat and other conditions are important factors in soil formation. The intensity and direction of microbiological processes, mineralization of organic matter largely depend on the agrophysical properties of the soil. The problem of optimizing the agrophysical properties of typical chernozem can be successfully solved by creating favorable parameters of structural composition and density in the root layer of the soil (Shevchenko et al., 2012). Studies of short-term crop rotations show that they improve soil fertility. This is due to the formation of a clumpy-grained structure and an increase in soil cohesion, water content, and better nutrient retention (Igbal et al., 2005; Zhang et al., 2011; Zhernova et al., 2022).

High saturation of crop rotation with sunflower has become the main issue of the Ukrainian agricultural sector today. Such an imbalance in the structure of crops can lead, first of all, to the depletion of moisture reserves in the root layer of the soil. The solution to this problem can be the establishment of a scientifically based interval for returning the culture to the crop rotation (Kharytonov et al., 2019).

The density and structural and aggregate composition of the soil are quite dynamic indicators that largely depend on weather conditions and many other factors. Soil response to density is different, so establishing patterns of its change in crop rotations with a short rotation, set and order of crop rotation is an important task of our research. Soil density affects the root system of crops in turn on the whole plant's physiological maturation, growth and yield, and additionally affects the nutrient intake, independent of the culture (Czyž et al., 2001; Wolkowski and Lowery, 2008; Lipiec et al., 2012; Nyéki et al., 2017). The aim of the research was to find out the effect of saturation of short-term crop rotations with sunflower on the structural and aggregate composition and density of soil compaction. And also, to find out the regularity of yield formation from agrophysical indicators on the studied variants.

MATERIALS AND METHODS

The study to determine the agrophysical parameters of typical chernozem was conducted on the basis of the chair of Farming named after O. M. Mozheiko of the experimental field of Kharkiv National Agrarian University named after V. V. Dokuchaev (KhNAU). The complexity of the climatic conditions of the Kharkiv region of Ukraine for agriculture is also revealed in not existing guaranteed annual sufficient moisture apart from it in certain years warm resources are much lower than are needed for crops. In addition, in some years, thermal resources are much less than the needs of crops. It is well known that precipitation affects soil compaction, which is a variable property.

The study by Nyéki et al. (2013) found that soil compaction as a factor of influence does not manifest itself in a direct way. However, they concluded that natural changes in soil compaction with changes in moisture (precipitation) for different soil types (first of all for different clay content %) can in some cases lead to significant differences in crop yields. According to the meteorological station of KhNAU, during the growing season of sunflower the average long-term precipitation was 278 mm, and the air temperature was +17.7°C. Precipitation in 2021 was 197.7 mm, which is 81.3 mm less than the long-term average, and the excess of the average daily air temperature by 2.5°C compared to the long-term average. Therefore, sunflower vegetation during this period took place in relatively unfavorable conditions. During the growing season of sunflower in 2022, precipitation was 97 mm above normal, and the average air temperature was 17.7°C, which is 1.7°C higher than the climatic norm.

The physical properties of the soil are affected by many factors that change vertically with depth, laterally across fields and temporally in response to climate and human activity (Debaeke et al., 2017). The soil cover of the experimental field is represented by typical chernozem heavy loam on loess-like loam. In terms of agrophysical and agrochemical properties, it is one of the most favorable soils for growing field crops. It is also characterized by high reserves of nutrients available to plants, high humus content and intense biological activity.

The arable layer of the soil (0-30 cm) contains humus (according to Tyurin) - 4.9-5.1%, easily hydrolyzable nitrogen (according to Kornfield) - 81 mg/kg of soil, mobile forms of phosphorus and potassium (according to Chirikov) - 100 and 200 mg/kg of soil. Content of exchangeable cations: calcium - 37.8%, magnesium - 6.6%, sodium - 0.49%, potassium - 0.5%, hydrogen - 21 mg-equiv./kg soil. The soil reaction - pH: aqueous - 7.0, salt - 5.2-5.6. Groundwater lies at a depth of about 18 m (Tykhonenko and Dehtiarov, 2016). Sunflower hybrid - Cruiser LG59580. The size of the sowing area is 750 m^2, the accounting area is 100 m^2. Variants of short-term (5-field) crop rotations with different proportions of sunflower in the structure of the sown areas were studied (Table 1).

Table 1. Crop rotation structure, %

Pea	Winter wheat	Corn	Winter rye	Fallow	Sunflower
20	20	20	20	–	**20**
20	20	–	20	–	**40**
–	20	–	20	–	**60**
–	20	40	20	20	**0**

The study was conducted by the following methods: the density of the composition of the intact soil by the method of the cutting cylinder according to N.A. Kaczynski (DSTU ISO 11272-2001); structural-aggregate composition and water resistance of structural aggregates by the method of fractionation on sieves according to M.I. Savvinov (DSTU 4744-2007).

RESULTS AND DISCUSSIONS

The density of soil composition is an important indicator of its agrophysical properties. This indicator plays a significant role in the growth and development of crops and the formation of the harvest. The optimal soil density for most crops is in the range of 1.0-1.3 g/cm^3. The given intervals are not constants. They vary over time and, above all, depending on soil moisture. With high moisture, the optimum shifts to lower values, and with insufficient moisture - to higher values (Birkas et al., 2009). Numerous studies have established the optimal parameters of soil density for growing sunflower in the Forest-Steppe zone of Ukraine on typical black soil - 1.0-1.1 g/cm^3.

The root system of agricultural plants mainly develops in the upper 0-30 cm layer of soil, which accounts for 80-90% of its total mass. It is important to know the generalized density of this soil layer, as well as its dynamics during the growing season. Porosity, water permeability, productive moisture reserves, root system growth, and plant development in general depend on the density of the topsoil (0-30 cm) (Yevtushenko et al., 2018).

In our research, we noted the regularity of soil density distribution by variant. These indicators increased from the upper to the lower soil layers. It should be noted that the crop rotation without sunflower and the crop rotation with a 20% share had similar values. Figure 1 shows the average results for two years (2021-2022) of the soil density study depending on the saturation of short-term crop rotations with sunflower. It was found that an increase in the share of sunflower in the crop rotation contributes to the compaction of the arable (0-30 cm) soil layer. Nevertheless, this soil layer had optimal values of compaction density for all variants, which were in the range of 1.09-1.14 g/cm^3. It was highest in the variant where sunflower occupied 40 and 60% of the crop rotation area - 1.13 and 1.14 g/cm^3. In crop rotations without sunflower and with a share of 20%, the density of the tilth layer was 1.09 g/cm^3.

The highest soil layer density of 0-10 cm was in the crop rotation with a share of sunflower of 40% - 1.11 g/cm^3. Gradually, it decreased to 1.09 and 1.07 g/cm^3 in the variants with sunflower saturation of 60 and 20%. Crop rotation without sunflower contributed to a decrease in soil density in the upper (0-10 cm) soil layer by 0.3 g/cm^3. In the soil layer of 10-20 cm, the soil density had optimal values, which ranged from 1.08-1.16 g/cm^3 in short-term crop rotations. It was highest in the crop rotation, where sunflower occupied 60% of the crop rotation area. The lowest values of this indicator were observed in the control variant of crop rotation and with a share of sunflower of 20%: 1.09 and 1.08 g/cm^3. Increasing the saturation of the crop rotation with sunflower to 40% led to a slight compaction of the soil layer of 10-20 cm by 0.04 g/cm^3 compared to the control. The density of soil compaction in the lower (20-30 cm) soil layer increased significantly in all variants. This soil layer was the most compacted in the crop rotation, where the share of sunflower was 60% - 1.17 g/cm^3. In the specified soil layer, the density decreased in the variants with 40 and 20% saturation of sunflower by 0.03 and 0.05 g/cm^3. Crop rotation without sunflower provided the lowest density of this soil layer at 1.10 g/cm^3.

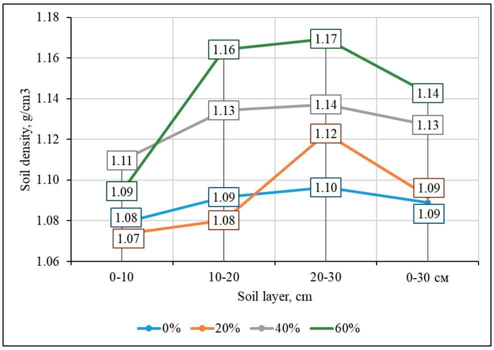

Figure 1. Density of soil composition, 2021–2022

Soil aggregation withstands soil fertility by reducing erosion and improving soil aeration, as well water retention. In addition, soil aggregation prevents soil organic matter from being mineralized by physically reducing the accessibility of organic compounds for microorganisms, and oxygen (Spohn and Giani, 2010).

The study of aggregates is one way to quantify whether management practices improve natural characteristics and agricultural capacity of soil. Soil aggregate and structure stability is the result of the interaction among many agents, such as the environment, management practices, crop, inherent soil features, and soil biological and non-biological processes (Bronick and Lal, 2005). The size of soil aggregate and stability are also used to characterize soil structure because those indicators are correlated with various soil functions (Rabot et al., 2018). Land use and associated management practices, such as crop sequencing, are the major and most direct ways of affecting soil structure and properties by their impact on destruction forces and aggregate-creating processes (Lehrsch et al., 2012).

Soil structure is defined as aggregates of various sizes and shapes that are glued together from soil particles and have the ability to break down. A structure with a lump size of 10-0.25 mm in diameter is considered particularly valuable. The higher its value, the longer the favorable conditions for the growth and development of crops are maintained, and the better the soil resists water and wind erosion. It is destroyed and restored under the influence of various factors: mechanical destruction, physical, chemical and biological processes. The soil structure is dynamic. Important measures aimed at maintaining the soil structure include a reasonable selection, ratio, and rotation of crops in crop rotation, etc. (Kudria et al., 2020).

The research results show that the number of soil structural aggregates depends on the share of sunflower in the structure of sown areas (Table 2). It was found that the structural composition of the tilth soil layer was almost the same across the experimental variants. The amount of agronomically valuable soil fractions with a size of 10-0.25 mm in the tilth layer of soil in the crop rotation with a share of sunflower of 20% was 64.2%. When the share of sunflower increased to 40 and 60%, the content of these aggregates decreased to 60.2 and 60.4%. The introduction of fallow in the crop rotation and the absence of sunflower resulted in an increase in the number of agronomically valuable aggregates to 66.0%.

Crop rotation with a 20% share of sunflower had a significant impact on the structural composition (78.5%) of the surface (0-10 cm) soil layer. Crop rotations without sunflower and with 40% saturation had a slightly worse impact. The number of soil aggregates in these variants was 9.9 and 16.9% less. The worst performance was in the crop rotation, where the share of sunflower was 60%. The average soil layer studied (10-20 cm) was characterized by almost the same aggregate content across variants and ranged from 61.8-63.3%. The only variant that stood out was the one with 60% sunflower saturation. The soil layer of 20-30 cm had an unequal number of aggregates of 10-0.25 mm in size. There was an increase in the number of lumps to 66.4% in the crop rotation without sunflower. The worst structural indicators were characterized by a plot under crop rotation with a share of sunflower of 20%. The quantity of structural particles in the bottom layer decreased by 15.5% compared to the previous variant. The structural parameters of typical chernozem under crop rotations with sunflower saturation of 40 and 60% did not differ significantly.

Table 2. Structural state of typical chernozem

Share of sunflower in crop rotation	Structural and aggregate composition of the soil (10-0.25 mm), %				Content of water-resistant soil aggregates (5.0-0.25 mm), %			
	2021-2022				2021-2022			
	0-10	10-20	20-30	0-30	0-10	10-20	20-30	0-30
0%	68.6	63.0	66.4	66.0	49.1	66.9	66.9	61.0
20%	78.5	63.3	50.9	64.2	62.2	84.0	86.7	77.6
40%	61.6	61.8	57.1	60.2	66.9	78.7	76.1	73.9
60%	47.4	72.2	61.8	60.4	68.7	74.1	73.3	72.0

Under the influence of short-term crop rotations, the content of agronomically valuable fractions of 0.25-5.0 mm in the arable 0-30 cm soil layer slightly changed. This figure was the highest in the second variant of the study (77.6%). There was a consistent increase in water-resistant aggregates with a depth of 62.2% (0-10 cm) to 86.7% (20-30 cm). This pattern did not occur for other variants. In the variant without sunflower, the content of aggregates in the soil layers 10-20 and 20-30 cm was at the same level. In the surface (0-10 cm) soil layer, there was a gradual increase in aggregates of 0.25-5.0 mm from 49.1

(control variant) to 68.7% (saturation of crop rotation with sunflower 60%). The content of water-resistant aggregates in the layers of 10-20 and 20-30 cm tended to decrease in accordance with the increase in the share of sunflower in crop rotations.

Typical untreated chernozem is characterized by the highest content of agronomically valuable water-resistant aggregates. Growing crops on this soil leads to dispersion of structural aggregates. Agrogenic chernozems contain four times less agronomically valuable (3-1 mm) water-resistant aggregates and twice as many aggregates < 0.25 mm in the 0-20 cm layer compared to virgin soil (Panasenko, 2015). Some studies on crop rotations show that the worst structure is characterized by a grain and fallow crop rotation. It has a structural coefficient of 3.4, which is 9.7 and 9.4% less than the grain crop rotation, respectively (K = 3.74 and 3.73). The deterioration of the soil structure in the grain and fallow crop rotation is due to the presence of a field of fallow and more intensive humus decomposition. The structure of crop rotations has a significant impact on improving the agrophysical properties of the soil. This leads to an increase in crop productivity, especially in dry years (Dehtiarov, 2013).

The results of Lebed et al. (1992) indicate the formation of better indicators of the structural state of the soil after growing crops of continuous sowing method, compared to row crops. They found that in crop rotations with a high proportion of row crops, soil structure deteriorates significantly. To avoid the negative effects of row crops on the soil structure, it is necessary that the period of their cultivation on the same field should not exceed three years.

This is confirmed by our research results. The influence of short-term crop rotations with different proportions of sunflower on some agrophysical parameters of typical chernozem in comparison with the control variant was revealed. The density of typical chernozem in crop rotation with a share of sunflower of 40 and 60% compared to the control is slightly worse, and the arable layer is compacted. The greatest compaction is in the subsoil layer of 20-30 cm, as a result of the formation of the plow sole. The density of the topsoil at 20% saturation with sunflower is at the level of the control variant.

Crop rotation without sunflower was characterized by the highest content of agronomically valuable aggregates - 66%. An increase in the share of sunflower in crop rotations is accompanied by a decrease in their number from 1.8 to 5.8%. The water resistance of the structure of typical chernozem was largely dependent on the cultivation of sunflower in crop rotations. In the control variant, the content of water-resistant aggregates ranged from 49.1% to 66.9%. In the variants of crop rotations with the inclusion of sunflower fields, there is an increase in the number of water-resistant aggregates both in the individual layers studied and in general in 0–30 cm. The highest water resistance of the soil structure was observed in the variant with 20% saturation of crop rotation with sunflower.

An average (0.48) correlation was found between sunflower yield and the density of 0-30 cm layer of the typical chernozem. There was no significant relationship between soil structure and yield.

CONCLUSIONS

Studies have shown that with different saturation of short-term crop rotations with sunflower, the topsoil was heterogeneous in terms of density and structural and aggregate composition. In crop rotation variants, the pattern of distribution of the zone between the upper (0-10, 10-20 cm) soil layers and the deeper compacted soil layer (20-30 cm) was manifested. In general, the structural condition of the tilth soil tends to improve. Namely, an increase in agronomically valuable fractions in the absence of sunflower in the crop rotation. The first variant of crop rotation, with a wider range of crops and the introduction of clean fallow, provided better agrophysical indicators of the tilth (0-30 cm) soil layer. It is worth noting the crop rotation with 20% sunflower, where the content of 10-0.25 mm fractions is only 2.2% lower than the control, and the density of the compound was on par with it.

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RESEARCH ON THE INFLUENCE OF HARVEST TIME ON YIELD IN GROUNDNUTS GROWN ON SANDY SOILS

Milica DIMA, Reta DRĂGHICI, Gheorghe COTEȚ, Florentina NETCU, Maria-Florentina BĂJENARU

Research Development Station for Plant Culture on Sands Dabuleni, 217 Petre Banita Street, Calarasi, Dolj, Romania

Corresponding author email: mariaciuca92@yahoo.com

Abstract

The research was carried out in the experimental field cultivated with peanuts at Research Development Station for Plant Culture on Sands in the period 2019-2021. The purpose of this study was to determine the effect of different harvest times on some elements of productivity and yield in peanuts grown under sandy soil conditions. The experiment was mono-factorial with three harvest periods: 153, 163, 173 and 183 days after sowing (DAS). The Viviana variety was used for sowing. The results showed that the number of mature pods per plant was higher in the late harvest. The pod yield over the three years increased from 3805 kg/ha to 4710 kg/ha when the harvest was delayed from 153 days after sowing (DAS) to 173 days after sowing (DAS).

Key words*: groundnuts, yield, pods, harvest, soil.*

INTRODUCTION

Peanuts (*Arachis hypogaea* L.) are considered one of the most important oil crops that occupy a world area of 25.2 million hectares with an annual world peanut production of 45.2 million tons (FAO, 2013). The groundnut crop is the second most important cultivated food and the world's fourth edible oil seed crop (Shilman et al., 2011). In the area of sandy soils in the south of Oltenia, peanuts find favourable pedological conditions for growth and fruiting, conditions that allow the cultivation of this species with good results (Mitrea I., 1993). In the context of climate change, crop production and food security in vulnerable areas are one of the major global challenges of the 21st century. Globally, agricultural systems are affected by climate variability and climate change that directly affect crop yields and indirectly biotic constraints and could lead to the invasion of weeds, pests and pathogens into areas where they were not previously relevant (Partal and Paraschivu, 2020; Paraschivu et al., 2021; Velea et al., 2021; Matei et al., 2021; 2022; Păunescu et al., 2022). Peanut seeds are an important source of proteins, lipids and fatty acids for the food industry. It is an important source of edible oil and protein for human consumption (Gulluoglu, 2011; Arioglu et al., 2013; Chamberlin et al., 2014; Chowdhury et al., 2015). Peanut kernels have an oil content of 47-50% (Sanders, 2002). They contain palmitic, oleic and linoleic acids, representing about 90% of the total fatty acids at maturity of the seeds (Young et al., 1972). Peanut seeds with high oleic acid provide a lower oxidation rate resulting in greater marketing acceptability (Mozingo et al., 2004). In 2015, peanut oil represented 3.0% of the world's production of vegetable oil (FAO, 2015). Peanuts are also a valuable source of vitamins E, K, and B (the richest source of thiamin and niacin) and other essential minerals (Kassa et al., 2009). Ishag (2000); Jordan et al. (2008) and Kaba et al. (2014) reported that peanuts have indeterminate growth types and that the plants produced many flowers during the growing period, but only 15-20% of the flowers produced mature pods. Young et al., (1982) reported that the total pod production increased continuously with the growing season, but that the harvested production decreased due to losses caused by the delay in the harvest date. Court et al. (1984) and Gulluoglu et al. (2016a) found that delayed harvesting increased yield, shelling yield and oil content. Sattayarak (1997) pointed out that harvest timings influenced yield, 1000 kernel weight, shelling yield, oil and protein content. Canavar and Kaynak (2013) reported that pod number and pod yield per plant, 1000-grain weight, shelling yield, oil and protein content increased by delaying harvest. Lu et al. (1997) reported that early harvesting resulted in the lowest pod and grain production, low oil and protein content. Wright and Porter (1991) and Kaba et al. (2014) showed that harvesting peanuts too early reduced production by 15%. Therefore, it is very important that harvesting is done at the right time to obtain a high yield and reduce production losses through seed germination in the soil. Oil content is an important quality characteristic for groundnut seeds.

The oil content of peanut seeds is influenced by genotype, growing conditions and maturity. Lu et al. (1997), Canavar and Kaynak (2013) showed that with the delay in harvesting time, the oil content of peanut varieties increased. The fatty acid content of peanut grains is influenced by genotype, sowing date, soil nutrients, air and soil temperature, growth and development conditions (Dwivedi et al., 1996; Isleib et al., 2008). Andersen and Gorbet (2002) and Gulluoglu et al. (2016a) reported that seed maturity can influence the fatty acid composition of peanuts. In general, oleic acid increases and linoleic acid decreases with seed maturity. The increase of oleic acid with seed maturity is normally accompanied by a decrease in palmitic and linoleic acids. Bovi (1982), Raheja et al. (1987) and Onemli (2012) reported that there was a negative correlation between oleic acid and linoleic acid.

Establishing the moment of harvesting for peanuts is particularly important, as it is necessary to place it so that it coincides with the achievement of the maximum number of mature pods per plant and the achievement of the minimum number of sprouted pods, knowing that in early varieties, without germination rest, the first seeds reached at maturity they sprout in the soil before harvesting (Krapovickas A., 1969, cited by Rehm S. and Espig G., 1976). The aim of this study was to investigate the effect of harvest data on some productivity and quality elements of peanuts grown on sandy soils in southern Oltenia.

MATERIALS AND METHODS

The paper presents the results of the research under the conditions of the Development Research Station for Plant Culture on the Dăbuleni Sands in the period 2019-2021, the variety used as plant material being the *Viviana* peanut variety. The research was carried out on sandy soil with low natural fertility, low in humus (0.38%) and rich in coarse sand (76%). The study was carried out in an irrigated system, with the water deficit during the period of growth and development being supplemented by irrigation. Climatic data during the growing season (2019-2021) compared to the multi-year average (1965-2021) were recorded. The experiment was located according to the method of randomized blocks, in three repetitions. The surface of the experimental variant was 6.3 m^2 (2.1 x 3.0 m).

The distance between rows was 0.7 m and the distance between plants per row was 0.18 m, giving a sowing density of 7.9 plants/m^2. Fertilization was done with 15-15-15 complex fertilizers before sowing and in vegetation at the beginning of flowering with ammonium nitrate. Weed control was done with Stomp 4 l/ha applied pre-emergence (immediately after sowing), in vegetation with 2 mechanical harrows and post-emergence herbicide with Fusilade 2 l/ha + Corum 0.25 l/ha. In order to complete the water deficit in the soil during the vegetation period, the culture was irrigated by sprinkling. Harvesting was carried out manually by pulling the bushes and then drying them in the sun, followed by a manual detachment of the pods, in four different moments with an interval of 10 days between them at: 153, 163, 173 and 183 days after sowing (DAS). At harvest, biometric determinations were made on some productivity elements (the number of pods/plant, weight of 1000 pods, peeling yield) and pod production at 9% humidity was determined.

RESULTS AND DISCUSSIONS

On the sandy soils at RDSPCS Dabuleni, during the experimental period 2019-2021, the average monthly air temperature during the vegetation period (April-October) was 12.7°C to 25.4°C in 2019, from 12.9°C to 24.9°C in 2020 and 9.7°C to 25.7°C in 2021. Recorded growing season precipitation was 289 mm in 2019, 347 mm in 2020 and 307 mm in 2021, but unevenly distributed (Table 1).

Table 1. Climatic elements registered at the Meteorological Station of RDSPCS Dabuleni during the peanut vegetation period (2019-2021)

Year/Month/ Climatic element	2019							2020							2021						
	IV	V	VI	VII	VIII	IX	X	IV	V	VI	VII	VIII	IX	X	IV	V	VI	VII	VIII	IX	X
Average air temperature, °C	12.7	17.4	23.4	23.8	25.4	20.2	19.5	12.9	17.7	22.0	24.5	24.9	21.0	13.6	9.7	17.6	21.7	25.7	24.6	18.3	10.0
Multiannual average temperature (1956-2021), °C	11.9	17.1	21.6	23.1	22.6	17.8	11.4	11.9	16.9	21.5	23.2	22.6	17.9	11.5	11.9	16.9	21.5	23.2	22.6	17.9	11.4
Rainfall, mm	53.4	55.4	87.2	54.8	12.0	10.0	16.2	11.6	59.2	55.8	73.0	51.0	40.2	56.2	30.6	55.0	53.0	16.8	9.0	9.2	133.0
Multiannual sum rainfall (1956-2021), mm	47.1	62.8	70.7	55.7	32.8	46.2	42.1	46.8	62.8	70.4	55.2	36.8	45.3	42.5	46.5	62.7	70.1	54.6	36.6	44.7	43.9

During the growing season, observations and determinations were made on the number of pods per plant, the weight of the pods per plant, and it was observed that the influence of the harvest season on the number of pods per plant and on the production of pods per plant, there are differences statistically significant. Young et al. (1982) and Canavar and Kaynak (2013) reported that the number of pods per plant increased by delaying the harvest time. The average number of pods per plant ranged from 16.9 to 22.2. It increased when the harvest was delayed from 153 DAS to 173 DAS, and then decreased when harvesting at 183 DAS. While the number of pods per plant was 16.9 pods when plants were harvested 153 DAS, it increased to 22.2 pods plants harvested 173 DAS and then the number of pods per plant was decreased to 20.3 pods when the harvest time was delayed to 183 DAS (Table 2).

Table 2. Influence of harvest season on average number of pods per plant and average pod yield per plant (2019-2021)

Harvest time	The average number of pods per plant	Pod weight per plant (g)
15 IX	16.9	39.9
25 IX	19.6	47.0
5 X	22.2	57.8
15 X	20.3	52.3
LSD 5% =	2.15	3.9

Depending on the harvest season, pod production per plant ranged from 39.9 to 57.8 g per plant. Pod production per plant increased when harvest time was delayed to 173 DAS. The highest pod production was obtained when the plants were harvested 173 DAS and after that, the pod production per plant started to decrease. As the harvest date was delayed, pod yield per plant increased from 39.9 g to 57.8 g per plant at 173 DAS and decreased to 52.3 g per plant when the harvest was achieved 183 DAS (Table 3).

Table 3. The influence of the harvest season on some productivity elements and the average production of pods per plant (2019-2021)

Harvest time	Weight of 1000 grains (g)	Shelling percentage (%)	Pod production (kg/ha)
15 IX	1,180	68.2	3,805
25 IX	1,220	69.3	4,550
5 X	1,252	70.5	4,710
15 X	1,270	71.3	4,605
LSD 5% =	231	1.84	202

Young et al. (1982) reported that total pod production increased continuously with increasing growing seasons, then decreased due to losses due to longer field sprouting. Delaying harvest after physiological maturity may result in many pods being left in the soil due to gynophores degradation. Similar results were reported by other researchers (Court et al., 1984; Knauft et al., 1986; Park and Oh., 1992; Lu et al., 1997; Rahmianna et al., 2009; Canavar and Kaynak, 2013; Kaba et al., 2014).

The mass of 1000 grains is one of the very important productivity elements for achieving production. The average values obtained were between 1,180-1,270 g. There was a difference in 1000-grain weight between harvest times averaged over the three years. By delaying the harvest time, the weight of 1000 grains increased significantly. The 1000-grain weight increased when the harvest time was delayed from 153 days after sowing to 173 days after sowing and the highest increase in 1000-grain weight (1,270 g) was found when the harvest was made at 183 days from sowing (October 15). Harvesting at physiological maturity gave better results than earlier harvesting for 1000-grain weight. Duncan et al. (1978) suggested that the length of pod filling period is correlated with 1000-grain weight. Therefore, it is very important to harvest at an appropriate time (the color of the mesocarp pod is brown or black) to obtain a weight of more than 1000 grains. Rahmianna et al. (2009) and Canavar and Kaynak (2013) reported that 1000-grain weight increased when harvest time was delayed. Differences between harvest periods were also for the percentage of peeling. The average values of the peeling percentage ranged between 68.2-71.3%. The peeling percentage increased when the harvest time was delayed, reaching the maximum level at the physiological maturity of the pods. Peeling percentages were higher at each subsequent harvest date. Overall, the shelling percentage increased from 68.2% for the first harvest date (153 days from sowing) to 71.3% for the October 15 harvest date (183 days from sowing). Knauft et al. (1986) and Canavar and Kaynak (2013) reported that the percentage of shelling increased when harvesting was delayed. Regarding the average production of pods obtained in the three years of experimentation, there was a statistically significant difference between the harvest periods. Thus, the average production of pods had values between 3,805 kg/ha and 4,710 kg/ha (Table 3). While pod production was 3,805 kg/ha when plants were harvested 153 days after sowing, it increased to 4,710 kg/ha in plants harvested 173 days after sowing, and then pod production decreased to 4,605 kg/ha when the plants were harvested 183 days after sowing.

CONCLUSIONS

The climate and soil conditions in the area of sandy soils in the south of Oltenia are favorable for peanut cultivation.

Peanut plants have an indeterminate growth type, and flowering and pod formation continue long into the growing season until close to harvest.

Establishing the moment of harvesting for peanuts is particularly important, as it is necessary to place it so that it coincides with the achievement of the maximum number of mature pods per plant and the achievement of the minimum number of sprouted pods, knowing that in early varieties, without germination rest, the first seeds reached at maturity they sprout in the soil before harvesting.

The number of pods per plant, 1000-grain weight, pod yield/ha, shelling yield increased when the harvest period was delayed. The maximum production of mature pods was obtained when it was harvested 173 days after sowing (October 5), after this date the production decreases due to the increase in the percentage of sprouted pods.

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AGRONOMIC POTENTIAL OF LEGUMES AND GRASS MIXTURES FOR RIPARIAN GRASSLANDS RENOVATION - FORAGE YIELD AND QUALITY

Niculae DINCĂ[1], Adrian Nicușor FILIP[1], Ana-Maria STANCIU[1], Andreea Antonia GEORGESCU[2], Corina Elena POPESCU[2], Daniela AVRAM[2], Cristina MIHAESCU[3], Lavinia BURULEANU[2], Daniel DUNEA[2]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Agriculture, 59 Marasti Blvd, District 1, 011464, Bucharest, Romania
[2]Valahia University of Targoviste, Campus, 13 Aleea Sinaia, 130004, Targoviste, Romania
[3] National University of Science and Technology Politehnica Bucharest, Pitesti University Center, 1 Targu din Vale Street, Pitesti, Romania

Corresponding author email: filip.ady@gmail.com

Abstract

The objective of this study was to evaluate the forage yield and chemical composition of six variants including two alfalfa cultivars, a bird's-foot trefoil cultivar, and three grass mixtures selected as valuable biological material for potential riparian grasslands' renovation. The experiments were carried out on plots in Dragomiresti village located near Dambovita River riparian area. One level of fertilization (120 g m^{-2}) was considered for each variant. The plant material had high concentrations of dry matter, crude protein, neutral detergent fiber, and phosphorus, respectively. All the fertilized samples exhibited higher concentrations of dry matter, nitrates, and phosphorus than the corresponding non-fertilized samples. High concentrations of water-soluble carbohydrates were determined for alfalfa which showed also the smallest amount of neutral detergent fiber. The maximum ADF content was found in the fertilized Valahia alfalfa cultivar and the non-fertilized complex grass mixture. Regarding the potential for riparian grassland renovation, we can conclude that the Valahia alfalfa cultivar and the complex grass mixture with five species performed better in terms of forage yield and overall quality.

Key words: *alfalfa, bird's-foot trefoil, grass mixtures, Dry matter, ADF, NDF, crude protein, renovation.*

INTRODUCTION

Assessing grassland productivity depends on a good knowledge of the interactions of environmental factors, vegetation type and properties, and human activity (Dunea and Dincă, 2014).
Vegetation settled spontaneously on many grasslands, being quite diverse due to their dynamic nature, having seasonal fluctuations in the dynamics of the species that make up that floristic composition, which generates various aspects during the vegetation period, depending on the phenology of the plants (Peter et al., 2008).
Annually, phenological changes occur in the vegetation of natural grasslands, depending on weather conditions, flowering periods, and other phenological phases, but a series of essential characteristics to define the phytocenosis, for example: the structure of the canopy, the floristic composition, the efficiency rates are more or less constant (Gosse et al., 1987).
The predominant vegetation of natural grasslands consists mostly of perennial plants. They are associated in complex functional groups that give grasslands specific features both regarding the relationships between the component species and between them and the biotope in which they are formed. Perennial species also show specific traits regarding economic value, improvement possibilities, and multifunctional use.
To address the new challenges arising from climate change and increasingly significant human disturbances, an increasing number of studies have been conducted on topics such as biodiversity conservation, grassland restoration, as well as the sustainable use and management

of grassland resources and the enhancement of their multifunctional role.
Among the 38 major themes identified by Zhao (2023), ecology and environment themes such as biodiversity conservation, land use and soil erosion, climate change, and paleoenvironment have developed rapidly. The increase in biodiversity conservation ranking during the study period (from 1900-) was the fastest.
The ranking of technology-related topics such as remote sensing and numerical modeling has also increased in recent decades. The ranks of traditional themes (e.g., biological nitrogen fixation, grazing, plant nutrition, germplasm and breeding, forage cultivation, and animal production) showed a sharp downward trend.
However, due to the climate variability impact on floristic composition, new experiments should be performed to support grassland renovation for pastoral value improvements (Dincă and Dunea, 2018).
The nutrient and biologically active content of forage is strongly related to animal health and welfare, thus having an impact on people's nutrition. The conversion of fodder into livestock production is one of the main agricultural production processes, and the problems of the rational utilization of fodder in animal nutrition acquire a key value (Cosman, 2018).
Various forages differ according to their technological properties, botanical composition, content of nutrients, and influence on the animal body.
The effectiveness of the feed will be higher the more it corresponds to the requirements of the domestic animals, in terms of physico-mechanical properties and content of nutrients, in agreement with their productive genetic potential (Cosman, 2018).
Apart from ensuring the traditional indices of food rations with protein, fat, cellulose, ash, calcium, phosphorus, carotene, the ratio between energy and protein, sugar and protein, the amount and correlation of amino acids, the amount and correlation of microelements, as well as of the vitamins are of great importance. In many cases, the evidence of these indices is decisive for increasing the assimilation of the essential nutritional elements of the basic forage (Cosman, 2018).
Increasing resilience through the renovation of the riparian grassland is one of the measures of implementing the direction of action "*Assessing the vulnerability of species and ecosystems to the effects of climate change*", sustaining the objective "*Supporting the development of a coherent, connected and representative network of protected areas which implements the adaptive management*" of The National Strategy on Adaptation to Climate Change for the period 2022-2030 with the perspective of 2050.
In this direction, the experimental approaches have considered the location of a field experiment for testing some mixtures of grasses, but also some valuable legumes in pure culture, in the riparian area or immediately close to the Dâmbovița River, monitoring the established pilot areas through in situ assessments, but also at the hydrographic basin level by using remote sensing means (case study - Argeș Hydrographic Basin).
This paper aims to characterize the agronomic potential of grass mixtures (three types of various complexity) and legumes (two alfalfa cultivars and 1 cultivar of bird's-foot trefoil) for riparian grassland renovation based on forage yield and quality.
The degree of novelty of our study lies in the fact that the renovation of riparian grasslands is not commonly studied despite the multifunctional valences of these areas and the selected biological material considered for screening is novel.

MATERIALS AND METHODS

The experiments were carried out on plots in Dragomiresti village located near Dambovita River riparian area, (Lat. N44°53'7.40", Long. E 25°24'6.89"). The soil type is luvic brown. For potential grassland renovation in riparian areas, we have selected species that almost naturally occur when proper conditions are met (Table 1). Alfalfa was considered with two valuable cultivars: Valahia - V1, a new Romanian variety (Dincă et al., 2022), and Italian Pomposa - V2, often cultivated by the farmers. Another legume was the bird's-foot trefoil (Leo cultivar) - V3. The grass mixtures were considered with three degrees of complexity: perennial ryegrass and red fescue - V4, perennial ryegrass, tall fescue, and smooth meadow-grass - V5, and red fescue, perennial ryegrass, smooth meadow-grass, tall fescue, and annual ryegrass - V6. One level of fertilization (120 g m^{-2}) was considered for each variant i.e., 16% (N) total nitrogen, 4.7% (N) nitric nitrogen, 11.3% (N) ammoniacal nitrogen, 5.2% (P_2O_5) phosphorus pentaoxide soluble in neutral ammonium citrate solution and water, 3.4% (P_2O_5) soluble phosphorus pentaoxide, 5.2% (K_2O) soluble potassium oxide, 3% (MgO) total magnesium oxide, 26% (SO_3) total sulfur trioxide, 4% (Fe) total iron.

Consequently, 36 plots were grouped into 6 randomized blocks, 3 fertilized and 3 unfertilized with 3 repetitions (6 × 2 × 3). The sowing was performed on 24 March 2023. Sampling of the vegetal material was performed on 27 June 2023 (94 DAS - days after sowing) using a quadrat of 50 × 50 cm in two points of each parcel on the diagonal. The harvested material was dried in an oven to assess the dry matter content.

Table 1. Biological material used in the experiment (percentage of participation in the seeding material)

V1	
MEDICAGO SATIVA VALAHIA	100%
V2	
MEDICAGO SATIVA POMPOSA	100%
V3	
LOTUS CORNICULATUS - LEO	100%
V4	
LOLIUM PERENNE 1	45%
FESTUCA RUBRA	25%
LOLIUM PERENNE 2	30%
V5	
FESTUCA ARUNDINACEA 1	37%
POA PRATENSIS	9%
LOLIUM PERENNE	9%
FESTUCA ARUNDINACEA 2	37%
FESTUCA ARUNDINACEA 3	8%
V6	
FESTUCA RUBRA	10%
LOLIUM PERENNE 1	25%
LOLIUM PERENNE 2	10%
LOLIUM PERENNE 3	5%
POA PRATENSIS	8%
FESTUCA ARUNDINACEA 1	3,90%
FESTUCA ARUNDINACEA 2	1,10%
LOLIUM MULTIFLORUM 1	4,60%
LOLIUM MULTIFLORUM 2	32,40%

The fresh samples were dried in an oven (SLW 53, POL-EKO, Poland) at 60°C for 72 h. The dried samples were then ground using a vibratory disc mill (Retsch RS 200, Germany) and sifted through a 1-mm mesh sieve.

The dry matter (DM) content was determined by drying the samples at 105°C for 3 h, until the constant mass. The ash content was measured by calcination at 550°C in a lab furnace (STC 411.06, Sweden). The crude protein (CP) content was calculated by multiplying the nitrogen content determined using the Kjeldahl method by 6.25. Water-soluble carbohydrates (WSC) were analyzed using the method with 3,5-dinitrosalicylic acid, measuring the absorbance of the solution at 540 nm (UV/VIS spectrophotometer UV 1720). Acid detergent fiber (ADF) was determined according to Van Soest et al. (1991) and ISO 13906:2008. Neutral detergent fiber (NDF) was determined using heat-stable amylase and sodium sulfite (Van Soest et al., 1991).

The nitrates content of the samples was determined spectrophotometrically by measuring the intensity of the color of the yellow-orange nitroderivatives resulting from the reaction of NO_3^- with phenol 2,4-disulfonic acid at 470 nm. The phosphorus content was calculated by multiplying with 0.436 the amount of the phosphoric anhydride determined spectrophotometrically, based on the formation of a colored complex with the ammonium molybdate, whose absorbance was read at 720 nm.

All the chemical assays were performed in triplicate and the results were expressed on DM basis.

The data were reported as the mean ± standard deviation and analyzed using SPSS 26.0 (SPSS, USA) for descriptive, comparative, and associative analyses.

RESULTS AND DISCUSSIONS

The **dry matter** (DM) is an important characteristic of the forage quality. It includes the macronutrients (i.e., proteins, carbohydrates, fat), but also the micronutrients (i.e., minerals, vitamins) and the compounds important in animal digestion (i.e., fibers). The preservation of the forage is strongly related to the amount of dry matter.

Figure 1 shows the dry matter accumulation at 94 DAS both for non-fertilized and fertilized variants. The dry matter content ranged between 95.2 g m^{-2} (V5NF) and 594.2 g m^{-2} (V6F). All the fertilized samples exhibited higher quantities of dry matter than the non-fertilized ones except the bird's foot trefoil (V3). It can be emphasized that within the tested variants, higher amounts of dry matter were determined in the fertilized samples V1F, V2F, V4F, V5F, and V6F compared to the non-fertilized ones.

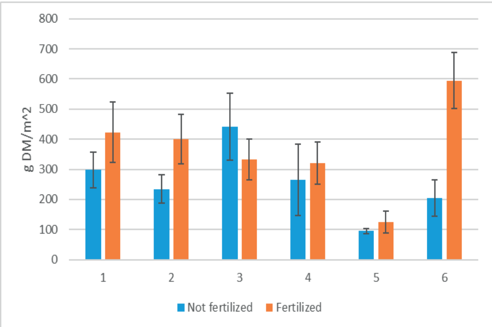

Figure 1. Dry matter accumulation for each variant at 94 DAS

Alfalfa cultivars and the V6 grass mixture presented significant DM gains following the fertilization.

The general image of the chemical data reveals differences between samples both in terms of the plant species and applied treatment (fertilized vs. non-fertilized).

The dry matter content of the experimental samples (Figure 2) ranged between 219.35 g/kg FW (V5NF) and 224.97 g/kg FW (V5F). Practically, the fertilization treatment had a higher influence on the dry matter content of the sample V5, determining the increase of this parameter with 2.56%. In the fertilized samples, an increase of the dry matter content was also determined in V6 with 1.56% comparatively with the non-treated variant.

The **ash** of the samples (Figure 2) was different within the group of the samples, being significantly greater in V4F (122.59 g/kg DM), while V6NF was situated to the inferior limit of the determined values (103.93 g/kg DM) for this parameter. If we discuss the fertilized samples *vs*. the unfertilized ones in terms of their mineral content, it can be remarked that all the experimental samples, except V1, had a smaller amount of ash than their corresponding fertilized variants. This aspect is important in the context in which no nutritional benefit to livestock is brought by high levels of ash content. The concentration of ash in the green mass (*Lotus corniculatus* 'Doru') was high at the third cut (10.66% DM), the smallest value, of 9.14% DM, being determined at the fourth cut (Cosman et al., 2020).

The ash content of the first-cut alfalfa cultivar Banat harvested at the initial flowering stage and wilted 24 h, until a dry matter of 419.9 g/kg, was 86.48 g/kg DM (Đorđević et al., 2016).

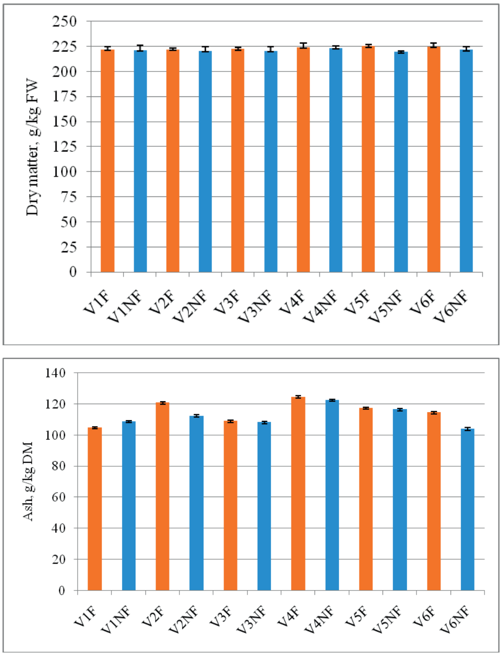

Figure 2. Dry matter content (a) and ash content (b) for each variant

The nutritive value of white clover (*Trifolium repens* L.) and Kentucky bluegrass (*Poa pratensis* L.) was evaluated by (Stojanović et al., 2022). The authors reported a significant effect of the consecutive cuts during the spring growing season on their quality. The DM of the white clover was 18.07% at the first cut, respectively by 20.93% at the third cut. The dry matter content of *Poa pratensis* ranged between 21.61% (cut I) and 22.42% (cut III), the value determined at the second cut being 22.43%.

The ash content of the same species ranged between 8.97% (first cut) and 8.70% (third cut) in the case of the legume, respectively between 7.12% (first cut) and 6.84% (third cut) for the grass herbage.

Forage nutritive value is directly related to the content of essential nutrients (McDonald et al., 2021), and being affected by different factors. It was reported that it depends on the year of cultivation, but also, even if they are cultivated in the same field, similar plants can exhibit varying nutritive values.

The quality of proteins is determined by their amino-acid composition. Alfalfa does not require nitrogen fertilization (Dien et al., 2006), its leaves being a valuable supplemental protein feed for livestock.

Excepting the V5 sample, in all the non-fertilized samples, higher quantities of **crude protein** (Figure 3) were determined than in the corresponding fertilized ones. The crude protein content ranged between 83.76 g/kg DM (V6F) and 190.61 g/kg DM (V4NF). Increased values of this nutrient, by 61.57% in V3NF than in V3F, respectively by 46.62% in V6NF than in V6F, were determined.

The crude protein content of the freshly chopped alfalfa wilted to 300 and 400 g DM/kg fresh weight, respectively, was determined by Zhang et al. (2021), recording values of 256 and 264 g/kg of DM respectively. Li et al. (2021) evaluated the chemical composition of fresh alfalfa with about 30% DM and determined an average value of 247 g CP/kg DM. According to Đorđević et al. (2016), the crude protein content of the first-cut alfalfa cultivar Banat harvested at the initial flowering stage and wilted 24-h was only 165.94 g/kg DM.

The CP concentration in cuts of two alfalfa and grasses as pure cultures and legume-grass mixtures was found from 65 to 199 g/kg DM (McDonald et al., 2021). Referring to this parameter, there was no interaction between species treatments and fertilizer applications in all cuts of two years.

The CP values for white clover and Kentucky bluegrass significantly decreased during the spring growing season (Stojanović et al., 2022). For clover, the CP content decreased from 26.25% DM (first cut) to 18.91% DM (third cut). CP of the second harvest of grass herbage, of 8.51% DM, was lower compared with the first and third ones.

An important factor that determines the quality and the feeding value of the forage is its content of sugar and starch as easily assimilable energy sources.

Alfalfa is typically difficult to ensile due to a low **water-soluble carbohydrates** (WSC) (Figure 3) concentration at harvest (Li et al., 2021). The concentration of WSC was only 18.37 g/kg DM in V6F, while an amount of 1.94 times higher was determined in the V6NF variant. Within the experiment, V1NF, V2NF, and V3NF exhibited a content of WSC greater than 50 g/kg DM. The WSC content of the variants without fertilization was higher comparatively with those of the fertilized samples, except the V5NF sample, as it has been determined for CP content.

The concentration of WSC was 45.9 and 46.2 g/kg of DM respectively in fresh chopped alfalfa wilted to 300 and 400 g dry matter/kg fresh weight (Zhang et al., 2021). An average value of 62.5 g WSC/kg DM was determined by Li et al. (2021) before the ensiling of fresh alfalfa wilted naturally to 312 g DM/kg FW.

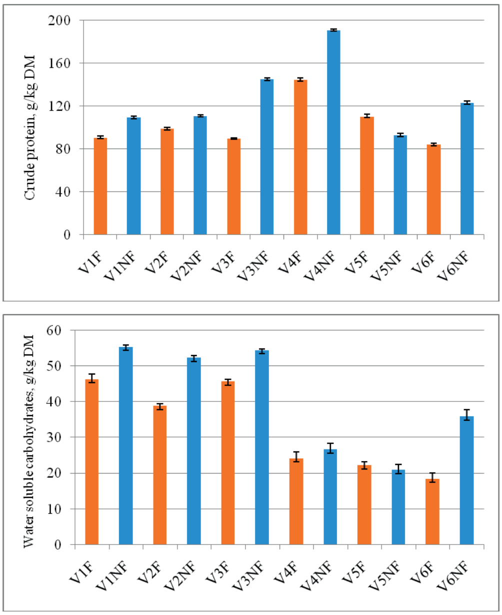

Figure 3. Crude protein content and water-soluble carbohydrates for each variant

The chemical composition in various alfalfa varieties, namely six species commonly found in northern China, was significantly different (Jianyu et al., 2023). The wilted forages were characterized by a dry matter content from 463.66 to 513.53 g/kg FM. The crude protein content ranged between 166.62 and 222.34 g/kg DM, while the WSC was found within the interval 38.37 and 66.06 g/kg DM.

Three forage species, namely alfalfa, reed canarygrass, and switchgrass were evaluated at different maturity stages by Dien et al. (2006) for their bioconversion potential as energy crops. The compositional analysis revealed that the concentration of ash and protein declined with the maturity of all three herbaceous species. Thus, the ash content of alfalfa bud was 81 g/kg DM, while the full flower showed 58 g/kg DM. The crude protein content ranged between 127 g/kg DM (alfalfa bud) and 88 g/kg DM (alfalfa full flower). The vegetative reed canarygrass had the highest concentration of crude protein, 88 g/kg DM, with this concentration decreasing to 45 g/kg DM in ripe seeds. The ash content of vegetative reed canarygrass was the highest of all the three analyzed biomass species, with 128 g/kg DM. The pre-boot switchgrass contained 65 g crude protein/kg DM and 89 g ash/kg DM.

The quality of the green mass of bird's-foot-trefoil cv. Doru varied depending on the harvest time (Cosman et al., 2020), as follows: crude protein 143.5-194.5 g/kg DM, soluble sugars 37.5-61.0 g/kg DM, and phosphorus 2.2-3.3 g/kg DM. It was reported that the concentration of CP in the green mass was high after the second harvest and very low after the fourth harvest.

The fiber composition of forage influenced to a large extent its biological value (Bozhanska, 2020). Cellulose plays an important physiological role not only as a source of energy but also as a factor that ensures the normalization of the digestive processes (Cosman, 2018). Determination of the cell wall polysaccharides is of vital importance when animal nutrition is discussed. Numerous methods for the determination of different fractions (cellulose, hemicelluloses, lignin) are known, the detergent fiber system being widely applied.

The **neutral detergent fiber** (NDF) content of the experimental samples ranged between 250.51 g/kg DM (V1NF) and 377.65 g/kg DM (V4NF) (Figure 4). The **acid detergent fiber** (ADF) recorded the smallest value, 168.64 g/kg DM for the sample V2NF, while the highest amount (312.99 g/kg DM) was determined also in a sample without fertilization, namely V6NF (Figure 4). Values close to this upper limit of ADF content were determined in V1F, too.

The non-fertilized variants V3, V4, and V6 exhibited higher amounts of NDF and ADF than the corresponding fertilized samples, respectively. The acid detergent fiber (ADF) of the freshly chopped alfalfa wilted to two dry matter contents of approximately 300 and 400 g/kg fresh weight recorded values of 220 and 293 g/kg of DM respectively (Zhang et al., 2021). Average values of 222 g ADF/kg DM and 296 g NDF/kg DM were determined by Li et al. (2021) through analysis of the chemical composition of the fresh alfalfa wilted naturally to about 30% DM, while You et al. (2022) determined a percent of acid detergent fiber by 31.1% of DM in alfalfa with 47.8% DM and 41.6% of DM neutral detergent fiber.

The ADF and NDF content of the first-cut alfalfa cultivar Banat harvested at the initial flowering stage and wilted 24 h were 351.78 g/kg DM and 408.61 g/kg DM, respectively (Đorđević et al., 2016).

The ADF and NDF content of the six samples of alfalfa wilted in the field for 12 h ranged between 257.24 and 345.87 g/kg DM, and 364.79 and 419.94 g/kg DM respectively (Jianyu et al., 2023).

The concentration of ADF in cuts of two alfalfa and grasses as pure cultures and legume-grass mixtures recorded values of 309 g/kg DM (roundup ready RR tonica alfalfa) - 431 g/kg DM (conventional alfalfa + tall fescue), while the NDF concentration ranged between 460 g/kg DM (low-lignin Hi-Gest 360 LL alfalfa) and 614 g/kg DM (smooth bromegrass) (McDonald et al., 2021). The authors established that among the legume-grass mixtures and alfalfa pure cultures were no differences in ADF. Also, in all cuts, no significant effect of nitrogen application on ADF concentration was determined. On the contrary, nitrogen fertilizer application significantly increased the NDF (McDonald et al., 2021).

Testing two biofertilizers (Lumbrical and Lumbrex) on a grassland with bird's-foot-trefoil of 'Leo' cultivar (Bozhanska, 2020) determined a higher amount of NDF and ADF in the dry feed mass in the variants with soil treatment compared to foliar. The average values reported were 404.11 g/kg DM for NDF and 289.18 g/kg DM for ADF, respectively.

An increase in the fiber content (NDF and ADF) of white clover and Kentucky bluegrass was observed by Stojanović et al. (2022) during the spring grazing period. Thus, in the second cut, the highest content of NDF was determined with 36.06% in legume and 60.61% respectively in grass herbage. In the same cut, the ADF content was 29.03% (*Trifolium repens* L.) and 36.57% (*Poa pratensis* L.).

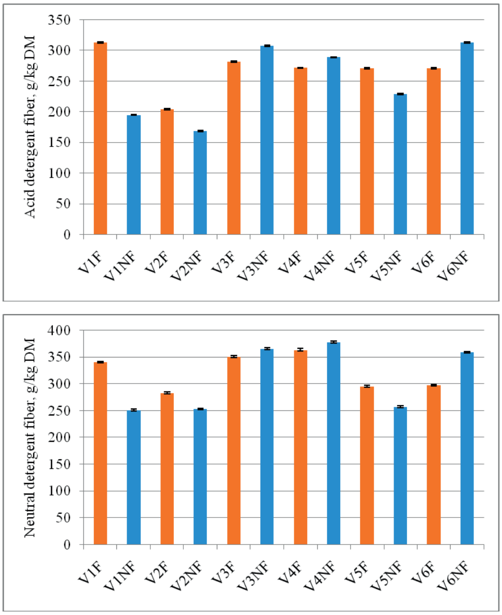

Figure 4. ADF and NDF content for each variant

The potential of the Romanian cultivars of tall fescue *Festuca arundinacea* 'Adela', 'Brio', and 'Măgurele 5' respectively for the restoration of degraded permanent grasslands in the Republic of Moldova was reported by Titei et al. (2019). The study of the quality of the freshly harvested biomass revealed that its dry matter contained 114-136 g/kg CP, 582-593 g/kg NDF and 392-396 g/kg ADF. The crude ash content ranged between 74 and 89 g/kg DM.

Nitrate is a naturally occurring form of nitrogen (Santamaria, 2006) that is formed from different sources, including fertilizers. The content of nitrate can vary within species and cultivars, the plants having different capacities to accumulate nitrate. This plant feature seemed to be correlated with the nitrate reductase activity, as well as to the degree of nitrate absorption and transfer in the plant (Santamaria, 2006).

The increasing use of synthetic nitrogen fertilizers and livestock manure in intensive agriculture raises the issue of the potential high content of nitrate in plants. Nitrate *per se* is relatively non-toxic, but part of the ingested nitrate is converted into the more toxic nitrite.

Because the nitrate metabolites are responsible for several health effects, the maximum levels for nitrates in foodstuffs (i.e., green leaf vegetables and baby food) are settled by the Commission Regulation (EU) 2023/915 of 25 April 2023 on maximum levels for certain contaminants in food.

The nitrate content was by far the highest in the sample V2, both in fertilized and non-fertilized variants. This parameter ranged in large limits within the set of samples, from 11.45 mg/kg DM (V5NF) to 110.93 mg/kg DM (V2F). Mean values were determined for V1F, V1NF, and V3F respectively, while the other samples contained smaller amounts of nitrates at the moment of the plant harvesting. In all the experimental samples, higher quantities of nitrates were determined in the fertilized samples compared to the non-fertilized ones.

Phosphorus fertilization increases plant yield, so it is compulsory to know the changes in the chemical composition of plants as they are affected by the level of fertilization. The content of phosphorus in samples ranged in rather lower limits compared to nitrates, i.e., between 215.89 mg/kg DM (V3NF) and 250.18 mg/kg DM (V4F), respectively. The amount of phosphorus in fertilized variants was 12.86% higher in V3F and only 3.32% higher in V5F. In all the experimental samples, higher quantities of phosphorus were determined in the fertilized samples compared to the non-fertilized ones. Fertilizer treatments with different ammonium nitrate doses increased the nitrogen and phosphorus content of three commonly cultivated grass varieties (*Lolium perenne* TOPGUN, *Festuca rubra* SERGEİ, and *Poa pratensis* AVALANCHE) (Alkan et al., 2022). The authors showed that the grasses absorb the phosphorus contents of the soil as a result of nitrogen-containing fertilization.

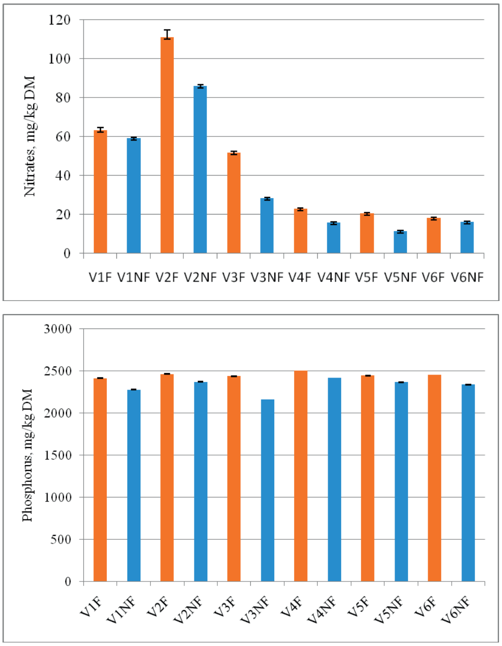

Figure 5. Nitrates and phosphorous content for each variant

A great impact of two growth stimulants (Ti and amino acids) on the chemical composition of *Poa pratensis*, including the phosphorus content, was also reported by Radkowski et al. (2022). Thus, compared to the control plants, the weighted average of P content ranged from 1.586 to 2.424 g/kg DM. A significant correlation between the dry matter yield and the P content was established.

The correlation between the crude protein content and the remanent concentration of the chemicals involved in increasing the plants' yield is mentioned below (Figure 6). The graphical representation emphasizes that a high amount of phosphorus is generally inversely correlated with crude protein concentration. The sample V4 (with and without fertilization) exhibited both high content of phosphorus and CP. If the nitrates level is discussed, the data scattering is large in the interval 0-40 mg nitrates/kg DM. Concentration of nitrates higher than 40 mg/kg DM led to average and small values of crude protein content.

The cumulative box plots of macronutrients (CP and WSC) and fibers (NDF and ADF) (Figure 7) emphasize the influence of fertilization on their concentration within the group of the experimental variants.

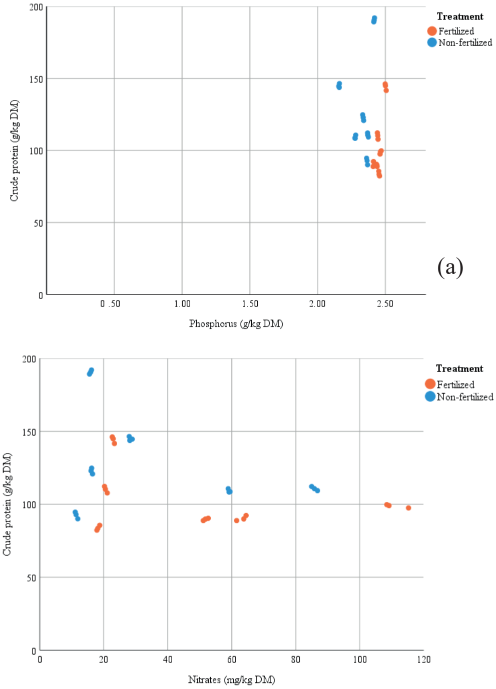

Figure 6. Scatter plots of the crude protein content and phosphorus (a), and nitrates (b)

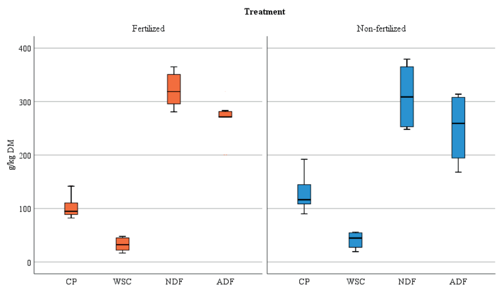

Figure 7. Box plots of CP, WSC, NDF, and ADF in experiments with and without fertilization

Thus, at the first cut of the plants, the fertilization type and level applied seemed to have a positive effect on the CP and WSC levels only in the case of V5, constituted from perennial ryegrass, tall fescue, and smooth meadow grass. To deepen the knowledge referring to this, a supplementary analysis is needed. A possible explanation is that at the moment of harvesting the entire process of transformation of the monomers (i.e. amino acids and monosaccharides respectively) under the action of the corresponding enzymes was not completed.

A different situation may emerge if the neutral and acid fibers are discussed. The fertilization treatment led to an increasing amount of fibers both in alfalfa cultivars and V5. On the contrary, V3, V4, and V6 exhibited a higher content of fiber in samples without fertilization (Figure 7).

One-way ANOVA analysis was applied to establish if the applied treatment determined significant effects on the dependent variables. The plant fertilization produced significant effects on the dependent variables i.e., DM, CP, and phosphorus.

Table 2. Pearson correlation coefficient values and type of association for the analyzed parameters; *correlation is significant at the 0.05 level (2-tailed); **correlation is significant at the 0.01 level (2-tailed)

-	Dry matter	Ash	CP	WSC	NDF	ADF	Nitrates	Phosphorus
Dry matter	1	0.245	0.034	-0.311	0.142	0.161	-0.132	**0.374***
Ash	-	1	**0.379***	**-0.612****	-0.029	-0.260	-0.073	**0.574****
CP	-	-	1	-0.058	**0.580****	0.267	**-0.345***	-0.184
WSC	-	-	-	1	-0.070	-0.210	**0.597****	**-0.610****
NDF	-	-	-	-	1	**0.855****	**-0.404***	0.011
ADF	-	-	-	-	-	1	**-0.589****	-0.050
Nitrates	-	-	-	-	-	-	1	0.094
Phosphorus	-	-	-	-	-	-	-	1

The correlation analysis (Table 2) shows an expected direct and strong correlation between NDF and ADF. An indirect correlation was established between WSC (a nutritional component) and ash content (that influences negatively animal nutrition), while between ash content and phosphorus, it was underlined also a moderate but positive correlation.

Weak and indirect correlations were established between (CP and nitrates) and (NDF and nitrates), respectively.

The Principal Component Analysis (PCA) was used to analyze the data for the dependent factors (Dunea et al., 2015). The relevant factor loadings (0.55) were considered for each component and the component relationships were plotted in Figure 8.

Table 3. Factor loadings (Varimax normalized) using the principal component extraction

Factor	Eigenvalue	Cum var (%)	DM	Ash	CP	WSC	NDF	ADF	NO3	P
Component 1	3.08	38.57	**0.789**	**0.688**	-0.091	**-0.855**	-0.014	0.032	-0.230	**0.889**
Component 2	2.35	67.96	0.286	-0.277	0.370	-0.223	**0.855**	**0.971**	**-0.713**	-0.126
Component 3	1.14	82.20	-0.011	0.634	**0.901**	-0.120	0.269	-0.093	-0.179	-0.107

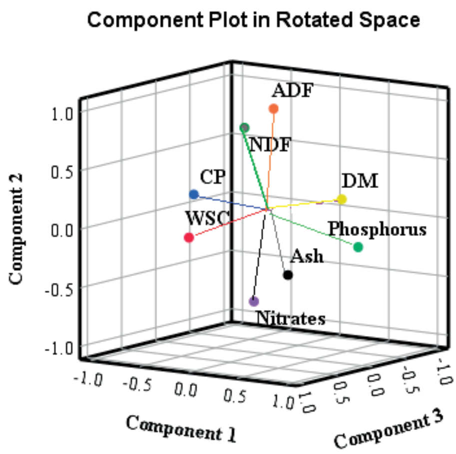

Figure 8. Relationships of components in PCA

Three PCs were extracted, which accounted for 82.20% of the total variance. PC1 explained 38.57% of the total variance, being formed by DM, ash, and phosphorus in its positive part, respectively WSC in its negative part. PC2 was highly positively associated with NDF and ADF and negatively with the nitrates concentration, while PC3 contained only the crude protein content (Table 3).

Hierarchical Cluster analysis allowed the grouping of the investigated parameters of the experimental samples into homogeneous

groups based on their common characteristics. This analysis intended to find out if, in the set of the eight investigated indicators of the forage, there are identifiable groups with similar characteristics.
The method used in clustering was the Ward method on variables of type interval, by applying the Squared Euclidean distance (Figure 9). The Hierarchical Cluster Analysis proved to be statistically significant at a significance threshold of 5%.

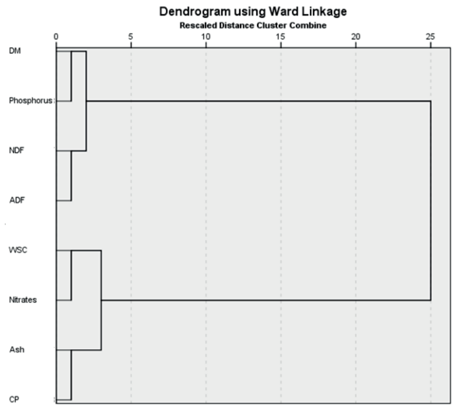

Figure 9. Dendogram of forage quality variables

The affiliation of each parameter to a certain cluster in the first stage of clustering was the forwards. Cluster 1: nitrates and WSC; Cluster 2: DM and phosphorus. In the second stage, ash aggregated to cluster 1 and NDF to cluster 2. CP, respectively ADF were involved in the third stage of clustering led to distinct two clusters.

The analysis of the classification of the indicators of forage quality based on their values determined in all 12 samples shows an optimum number of two classes. These clearly demarcate the forage macronutrients (CP, WSC), the nitrates potential involved in their increasing concentration, and the ash on the one part and dry matter, fibers, and the other compound by interest in fertilization (phosphorus), respectively.

Regarding the potential of tested biological material for riparian grassland renovation, we can conclude that the V1 (Valahia alfalfa cultivar) and V6 (the complex grass mixture with five species) variants performed better in terms of forage yield and overall quality, and thus further experiments should consider screenings of V1-V6 mixtures with various participations of alfalfa and grasses, and optimized levels of fertilization.

CONCLUSIONS

The forages must fulfill increased requirements, primarily regarding the concentration of nutrients and biologically active substances. Increased requirements are addressed not only to the amount of nutrients in forage but also to their quality and accessibility for the animal body. The nutrients' requirements can differ according to the degree of their utilization by various species of animals, and their physiological status.
According to our results, the nutritive value of grass mixtures was higher than legumes pure cultures. The stage of harvesting and the type of cultivar/type of plant mixtures should be carefully analyzed in relationship with the proposed fertilization scheme.
Further research will be conducted to extend the characterization of the plant material by determination of other chemical constituents and the digestibility of nutrients. The next experiments will consider legumes and grass mixtures with various participations based on the significance of the current results. Also, the correlation between the doses of fertilizers applied and the plant metabolism in terms of parameters of interest (i.e., for animal nutrition) will be on focus.

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THE BEHAVIOR OF SOME LOCAL AND FOREIGN COWPEA GENOTYPES IN THE CONDITIONS OF CLIMATE CHANGES IN THE SOUTH OF OLTENIA

Reta DRĂGHICI, Milica DIMA, Alina Nicoleta PARASCHIV, Maria Florentina BĂJENARU, Cristina BÎRSOGHE

Research Development Station for Plant Culture on Sands Dabuleni, 217 Petre Banita Street, Calarasi, Dolj, Romania

Corresponding author email: milicadima@yahoo.com

Abstract

*The research was carried out in the period 2021-2022 at the Research Development Station for Plant Culture on Sands Dăbuleni, Romania with the objective of the behavior of 16 cowpea genotypes (Vigna unguiculata L. Walp), in the conditions of sandy soils in the south of Oltenia. The obtained results highlighted the specificity of the cowpea for thermohydric stress, the plant tolerating very well the specific microclimate created by the recent climate changes. Thus, the tested cowpea genotypes recorded at plant maturity a number of 7.7-15.7 pods/plant and grain production values in the range of 1413.5-3065.5 kg/ha, highlighting a distinct correlation significant between the two components (r = 0.703**). The cowpea lines 27-B-3a, 27/2 and 25-A1-3 stood out, with the highest grain productions (2928.6-3065.5 kg/ha).*

Key words*: sandy soil, cowpea genotypes, physiology, productivity, quality.*

INTRODUCTION

Native to Africa, cowpea (*Vigna unguiculata* L. Walp.) is a diploid annual crop (2n = 22), herbaceous, predominantly self-pollinated (approx. 95%) that belongs to the *Leguminosae* family (Boukar et al., 2020), being one of the most important leguminous crops, because its seeds are rich in proteins, minerals and vitamins, components that give high value to food and feed (Sombié et al., 2018; Karuwal et al., 2021). The cowpea is considered to be the "queen of areas with psamosols", because through the biological properties of the plant it can promote a system of sustainable agriculture in areas affected by drought and with poorly productive lands, the plant making good use of the ecological conditions of the area of sandy soils (Zăvoi, 1967; Bashiru et al., 2018; Draghici et al., 2018).

The economic importance of the cowpea is highlighted by the plant's valuable properties, namely: resistance to drought, the particular contribution to increasing the content of organic matter in the sands, the symbiotic fixation of atmospheric nitrogen, a good precursor in crop rotation and, last but not least, important source of protein for the population in disadvantaged areas (Marinica, 1994; Matei et al., 2015; Draghici, 2018; Ciurescu et al., 2020). The quality of the production, highlighted by a rich content of essential plant-type nutrients such as carbohydrates, fibers, minerals and vitamins, recommends cowpea as a healthy, low-cost alternative/supplement to animal proteins (Nunes et al., 2022; Ciurescu et al., 2022).

Due to its special biological and morphological characteristics (very strong root system, with a high absorption power, waxy layer on the leaves), the cowpea can make good use of poorly productive lands in the category of sandy soils, having a high tolerance to thermal and water stress conditions (Rivas et al., 2016; Omolayo et al., 2021; Sánchez-Navarro et al., 2021). In recent climate change, drought has become a limiting factor for a wide range of crops, especially in temperate regions, as a result of high evaporation and evapotranspiration processes throughout the year (Körner et al., 2010; Beshir et al., 2016; Bonea & Urechean, 2020; Draghici et al., 2022). In this sense, it should be borne in mind that the development of plants and implicitly their production in a certain area is the result of the complex interaction between climatic and edaphic factors (Gheorghe et al., 2009; Bonea, 2020; Partal & Paraschivu, 2020). Compared to other leguminous plants, such as the bean (*Phaseolus vulgaris* L.), the cowpea is a plant with low requirements for water and nutrients, being successfully included in a farming system specific to sandy soils, which ensures profitability and protection the environment (Costa et al., 2011; Paraschivu & Cotuna, 2021).

MATERIALS AND METHODS

The research was carried out in the period 2021-2022 at the Research and Development Station for Plant Culture on Sands Dabuleni, Romania, with the objective of the behavior of sixteen cowpea genotypes (*Vigna unguiculata* L. Walp) in the conditions of the sandy soils of southern Oltenia, among which eleven Romanian genotypes and five genotypes from China. The experiment was organized according to the randomized block method in three repetitions, under irrigation conditions, on a sandy soil with low natural fertility, poorly supplied in total nitrogen (0.03-0.06%), well supplied in extractable phosphorus (47.1-79 ppm), low to medium supplied in exchangeable potassium (58.7-94.1 ppm) and with a moderately acidic soil reaction (pH_{H2O} =5.92-6.55), values within the fertility limits of soils from Romania, established by Davidescu et al. (1981).

The cowpea genotypes were sown in the first decade of May, at a distance of 70 cm between the rows, ensuring a density of 20 germinable seeds/m^2. The nutrition regime of the plant was achieved by fertilization with $N_{60}P_{60}K_{60}$. During the vegetation period, the following determinations were made: biology, biometrics, physiology and productivity of the plant, as well as the quality of production at harvest. In the flowering phase of the plant, the leaf surface was determined using the Area Metter 300 device and the physiological processes of the plant, respectively: photosynthesis rate, transpiration rate, stomatal conductance, with the LC Pro SD device.

The chlorophyll content of the leaf was determined with the CCM 200 Plus device. At harvest, the quality of the cowpea (protein and fat) was analyzed by the Perten method. The results were calculated and analyzed by the method of analysis of variance (ANOVA) and with the help of mathematical functions.

RESULTS AND DISCUSSIONS

The evolution of the climatic conditions recorded during the cowpea vegetation period (May-August) highlighted the increased drought phenomenon compared to the multiannual average temperature, by increasing the air temperature by 1.14 ^{0}C and recording a precipitation deficit of 84.81 mm (Table 1).

Table 1. Climatic conditions recorded at the weather station* of RDSPCS Dabuleni during the growing season of cowpea

Climate conditions 2021-2022	May	June	July	August	May-August
Average temperature (^{0}C)	17.95	22.3	25.45	24.85	22.64
Minimum temperature (^{0}C)	1.2	10.2	12.5	9.8	1.2
Maximum temperature (^{0}C)	31.8	39.6	41.6	41.2	41.6
Rainfall (mm)	46.7	50.8	15.9	29.2	142.6
Relative air humidity (%)	58.8	65.75	52.9	53.3	57.69
Multiannual average temperature (1956-2021) (^{0}C)	17.65	21.73	23.61	22.99	21.5
The sum of multiannual precipitation (mm)	63.26	70.47	55.06	38.62	227.41
Deviation of the 2021-2022 average temperature from the multiannual average temperature	0.3	0.57	1.84	1.86	1.14
Deviation of precipitation 2021-2022 compared to multiannual precipitation	-16.56	-19.67	-39.16	-9.42	-84.81

**AgroExpert from Adcon Telemetry SRL Romania*

The temperature regime and that of precipitation, in interaction with the very low relative humidity of the air, with values in the range of 52.9-65.75% and an average of 57.69%, generated an arid microclimate in the area of sandy soils in the south of Oltenia. In these conditions, the cowpea is an alternative to soybean and bean crops in areas with sandy soils, as a result of the resistance to drought due to both the very strong root system, with a great absorption power, and the waxy layer on the leaves and the number of stomata in the leaf which varies between 280-327 stomata/mm^2 on the upper face (Zăvoi, 1967; Matei et al., 2015).

Although it is a plant with good tolerance to drought, the yield of the cowpea plant can be affected, when soil water stress occurs during the reproductive stage of the plant (Oliveira et al., 2017; Moussa et al., 2021; Omolayo et al., 2021). In order to avoid the negative effects of water stress, it was necessary to supplement the water requirement by applying 2-3 waterings, with norms of 250 m^3 water/ha, during the period of flower bud formation and pod formation.

From germination to the end of the vegetation period, all the vital processes of the cowpea plant took place under high temperature conditions, over 10^0C.

During 2021-2021, the growing season of the sixteen bean genotypes lasted 82-97 days, with a heat requirement of 1,858.8-2,241.4^{0}C, depending on the genotype (Table 2).

The relations of plants with the heat factor are manifested starting with the germination phase of the seeds, and by the way the temperatures are directed in the first phases of vegetation, the growth and development periods of the plant and the level of production are influenced (Rățoi et al., 2014; Bonea, 2020).

The earliness of the plant is an important objective of the creation of varieties in areas subjected to aridification, in order to avoid periods of drought from the moment of flowering of the plants (Burzo, 2014; Bashiru et al., 2018).

Compared to the control variety, *Jiana*, all the cowpea genotypes taken in the study showed earliness in the range of 5-11 days, in 2021 and 8-20 days, in 2022.

The increase of 0.5^{0}C in the average air temperature for the period May-August 2022, compared to the same period in 2021, led to the registration of an early ripening of the pods, between 6-22 days, depending on the genotype.

Table 2. Analysis of the vegetation period recorded for cowpea genotypes in the climatic conditions of southern Oltenia

No. crt.	Genotypes	Year 2021		Year 2022		Average 2021-2022		Deviation 2022 from 2021	
		Vegetation period (days)	Thermal resources (∑^{0}C)	Vegetation period (days)	Thermal resources (∑^{0}C)	Vegetation period (days)	Thermal resources (∑^{0}C)	Days	(∑^{0}C)
1	Jiana	100	2,304	93	2,178.8	96.5	2,241.4	-7	-125.2
2	31-E2-2a	95	2,172	85	1,986.6	90	2,079.3	-10	-185.4
3	27/2	93	2,120	76	1,756.7	84.5	1,938.35	-17	-363.3
4	27-B-3a	93	2,120	84	1,968.2	88.5	2,044.1	-9	-151.8
5	32-B-3a	91	2,071	85	1,977.5	88	2,024.25	-6	-93.5
6	27-A4	92	2,096	77	1,719.4	84.5	1,907.7	-15	-376.6
7	25-A1-3	89	2,017	80	1,792.8	84.5	1,904.9	-9	-224.2
8	25-A2-4	91	2,071	79	1,775.1	85	1,923.05	-12	-295.9
9	25-B1-3	94	2,145	85	1,986.6	89.5	2,065.8	-9	-158.4
10	25-D1-4	97	2,226	75	1,674.5	86	1,950.25	-22	-551.5
11	25-D1-5	90	2,043	76	1,674.5	83	1,858.75	-14	-368.5
12	China T1	91	2,071	76	1,692.9	83.5	1,881.95	-15	-378.1
13	China T2	91	2,071	73	1,664.5	82	1,867.75	-18	-406.5
14	China T3	96	2,199	75	1,719.4	85.5	1,959.2	-21	-479.6
15	China T4	91	2,071	77	1,775.1	84	1,923.05	-14	-295.9
16	China T5	92	2,096	76	1,756.7	84	1,926.35	-16	-339.3
Average		92.9	2,118.3	79.5	1,818.7	86.2	1,968.5	-13.4	-299.6
Minimum		89	2,017	73	1,664.5	82	1,858.8	-22	-551.5
Maximum		100	2,304	93	2,178.8	96.5	2,241.4	-6	-93.5

The growth and development of cowpea plants was differentiated according to genotype (Table 3). Thus, average plant height values between 55.1-122 cm were recorded, with a maximum in the *Jiana* variety (control), compared to which all other genotypes recorded height differences statistically assured as distinct and very significantly negative.

Also, the leaf surface index recorded lower values in all studied genotypes, compared to the control variety, the differences being significantly and distinctly significantly negative. The productivity of the plant was highlighted by the number of pods, which varied between 7.7-15.7 pods/plant, with 8.5-10.9 grains in the pod and a pod length of 11-16.7 cm. Regarding the formation of pods per plant, from the assortment of cowpea genotypes tested, high values were recorded, between 12.6-15.7 pods/plant for the genotypes *27-B-3a*, *27/2*, *27-A4*, *25-D1-5*, which significantly and distinctly outperformed the *Jiana* variety.

Similar results were obtained by Pandiyan et al. (2020), by cultivating 28 cowpea genotypes in the Vellore region, India, highlighting a very high variability of the plant's morphological and productivity characters.

Photo 1. Jiana variety

Photo 2. Genotypes *25-A1-3* and *27-B-3a*

The leaf surface index correlated significantly positively with the height of the plant and significantly negatively with the number of pods/plant (Figure 1), which confirms that a luxuriant leaf apparatus prevents the penetration of sunlight at the level of the flower vexil, an essential condition in the process of pollen fertilization and implicitly of the plant's productivity. Research conducted in southern China on a number of 41 cowpea genotypes sown at different dates revealed that the development and erect bearing of the plant play a decisive role in the obtained bean production (Gong et al., 2023).

Table 3. Variability of some growth and development parameters of the cowpea plant, depending on the genotype

No	Genotypes	Plant height (cm)	Leaf surface index	No. Pods/ plant	No. Seeds/ pods	Pod length (cm)
1	Jiana (control)	122.0	8.8	7.7	9.1	14.6
2	31-E2-2a	63.4^{000}	4.5^{00}	10.5	9.9	14.8
3	27/2	70.5^{000}	5.1^{0}	13.1*	9.0	12.6
4	27-B-3a	53.4^{000}	4.3^{00}	15.7**	9.3	11.0^{0}
5	32-B-3a	53.9^{000}	5.2^{0}	8.7	8.9	16.2
6	27-A4	66.1^{000}	4.9^{0}	12.8*	8.7	16.1
7	25-A1-3	54.8^{000}	4.5^{00}	11.2	8.5	13.0
8	25-A2-4	54.5^{000}	4.3^{00}	10.2	9.9	13.8
9	25-B1-3	66.5^{000}	4.1^{00}	9.3	9.9	12.6
10	25-D1-4	78.0^{00}	4.6^{00}	11.0	9.6	12.4
11	25-D1-5	36.5^{000}	4.8^{0}	12.6*	9.5	12.6
12	China T1	38.0^{000}	3.8^{00}	9.6	10.1	16.7
13	China T2	33.5^{000}	3.9^{00}	9.3	10.9*	16.6
14	China T3	27.5^{000}	4.0^{00}	8.3	9.1	13.8
15	China T4	32.7^{000}	5.0^{0}	10.9	9.6	13.1
16	China T5	30.5^{000}	4.3^{00}	8.1	8.5	12.4
	LSD 5%	23.4	3.0	4.3	1.6	2.8
	LSD 1%	32.4	4.1	5.9	2.2	3.9
	LSD 0.1%	44.7	5.7	8.2	3.0	5.4

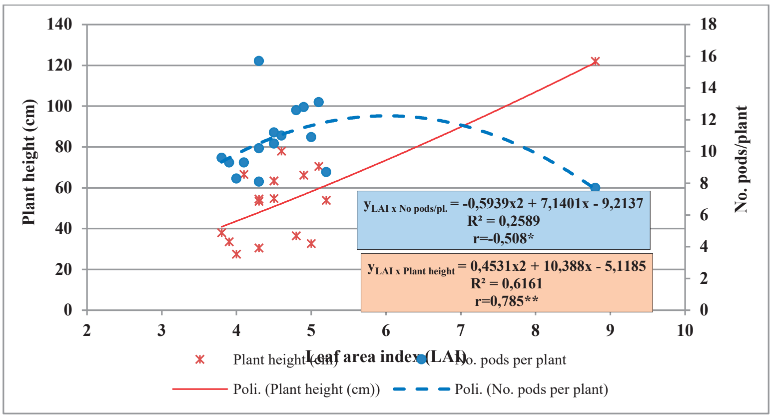

Figure 1. Correlations between leaf area index with plant height and number of pods/plant in 16 cowpea genotypes

The results regarding the physiology of the cowpea plant revealed a diurnal variation of the physiological processes of photosynthesis, transpiration and stomatal conductance, closely related to changes in environmental factors, especially solar radiation and temperature, recorded at leaf level with the LC Pro SD device (Tables 4 and 5).

Table 4. Climatic conditions at the leaf surface determined with the LC Pro SD device

Climatic conditions	9 o'clock			11 o'clock			11 o'clock		
	Average	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum
Solar radiation active in photosynthesis ($\mu mol/m^2/s$)	1,567.1	1,530	1,590	1,972.4	1,940	2,006	1,979.5	1,911	2,035
Temperature (^0C)	30.0	25	32.3	35.4	32	37.1	38.9	36.6	40.6
Atmospheric pressure (hPa)	1,019	1,019	1,019	1,019.1	1,019	1,020	1,017.4	1,017	1,018

Table 5. Diurnal variation of physiological processes recorded in cowpea genotypes

Cowpea genotypes	Plant photosynthesis rate ($\mu mol\ CO_2/m^2/s$)			Plant transpiration rate ($mmol\ H_2O/m^2/s$)			Stomatal conductance ($mol/m^2/s$)			Chlorophyll (CCI units)
	9 o'clock	11 o'clock	15 o'clock	9 o'clock	11 o'clock	15 o'clock	9 o'clock	11 o'clock	15 o'clock	9 o'clock
Jiana	20.99	11.93	5.16	1.50	2.26	0.73	0.13	0.10	0.02	45.30
31-E2-2a	24.20	20.95	16.63	2.64	5.16	5.52	0.17	0.27	0.22	78.20
27/2	20.26	26.63	21.52	3.22	5.89	6.27	0.22	0.34	0.24	65.90
27-B-3a	25.13	22.72	23.11	4.47	5.86	6.97	0.35	0.31	0.28	52.30
32-B-3a	23.89	24.00	22.68	3.61	6.31	7.47	0.23	0.35	0.32	53.77
27-A4	17.83	24.56	19.27	3.28	6.44	6.91	0.21	0.34	0.25	63.20
25-A1-3	20.08	25.45	14.58	3.11	6.52	8.20	0.19	0.33	0.33	54.70
25-A2-4	22.40	22.64	14.27	3.58	6.84	6.38	0.22	0.34	0.19	55.63
25-B1-3	25.96	25.34	13.83	4.19	7.10	6.54	0.29	0.34	0.19	73.53
25-D1-4	18.81	26.82	20.24	2.69	7.52	7.86	0.14	0.38	0.26	75.23
25-D1-5	13.68	10.98	12.64	2.85	4.38	5.45	0.15	0.14	0.15	82.47
China T1	28.03	22.09	8.49	4.16	8.65	5.23	0.26	0.47	0.13	72.07
China T2	17.70	22.94	15.37	2.99	6.73	6.85	0.15	0.30	0.19	58.17
China T3	13.87	14.66	21.25	2.40	4.76	8.82	0.11	0.18	0.25	74.20
China T4	13.96	23.36	17.40	3.66	6.65	9.81	0.20	0.30	0.34	38.60
China T5	19.79	20.92	15.48	3.81	7.25	8.95	0.22	0.35	0.30	47.73
Average	*20.41*	*21.62*	*16.37*	*3.26*	*6.14*	*6.75*	*0.20*	*0.30*	*0.23*	*61.94*
Minimum	*13.68*	*10.98*	*5.16*	*1.50*	*2.26*	*0.73*	*0.11*	*0.10*	*0.02*	*38.60*
Maximum	*28.03*	*26.82*	*23.11*	*4.47*	*8.65*	*9.81*	*0.35*	*0.47*	*0.34*	*82.47*

The photosynthesis process recorded differentiated values according to the cowpea genotype, with a daily average of 20.41 $\mu mol\ CO_2/m^2$/second at 9 o'clock, 21.62 $\mu mol\ CO_2/m^2$/second at 11 o'clock and 16.37 $\mu mol\ CO_2/m^2$/second at 3 p.m., when the temperature and radiation active in photosynthesis, recorded at leaf level, showed maximum values (temperature = 36.6-40.6^{0}C; radiation active in photosynthesis = 1,911-2,035 $\mu mol/m^2$/ second. *Jiana, 31-E2-2a, 25-B1-3* and *China T1* cowpea genotypes, recorded a downward trend of CO_2 assimilation throughout the day, with a minimum at 3 p.m., while regulating the plant's transpiration process by reducing water losses at leaf level. Some cowpea genotypes (*27-B-3a, China T3*) maintained their intensive photosynthesis process even at temperatures above 40^{0}C, but the optimal microclimate conditions for the development of physiological processes in most genotypes were achieved at 11 o'clock, when temperatures of 32-37.1^{0}C, a solar radiation of 1,940-2,006 $\mu mol/m^2$/second and an atmospheric pressure of 1,019-1,020 hPa were recorded.

Regarding the chlorophyll content index (CCI), determined at 9 o'clock with the CCM 200 Plus device, it showed differences according to the analyzed genotype, with the maximum in the *25-D1-5* genotype (CCI = 82.47) and the minimum in the *China T4* genotype (CCI = 38.6). Similar results for chlorophyll content were obtained in South Africa by Gerrano et al. (2022), on a number of 20 cowpea genotypes originating from South Africa, Nigeria and Kenya, obtained from the gene bank collections of the Agricultural Research Council - Vegetables, Industrial and Medicinal Plants (ARC-VIMP), showing values of CCI = 6.23-87.97. The physiological and metabolic changes are due to the differential accumulation in the root and leaves of metabolites, in particular proline, galactinol and a quercetin derivative, which can significantly influence a progressive acclimatization of the cowpea plant to stress stress during the day (Goufo et al., 2017). The degree of stomatal opening is an important indicator of plant response to thermohydric stress. Stomatal closure is an important drought tolerance mechanism, and cowpea can be considered a conservative species, that is, one that prioritizes the maintenance of water status rather than the photosynthetic rate (Oliveira, 2017).

The results obtained in our experiment revealed distinctly significant positive correlations between stomatal conductance and photosynthesis and transpiration processes recorded in cowpea (Figure 2). The rate of carbon penetration into plant increased with the increase in the degree of opening of the stomata, the relationship being established by a polynomial equation of the end degree, whose correlation coefficient is positive.

Extrapolating the functional connection between the degree of stomatal opening and the transpiration process, an upward trend of water loss was highlighted, along with the increase in stomatal conductance values. Similar results, which showed that the decrease in photosynthetic rate of cowpea plant could be attributed to the decrease in stomatal conductance observed under drought stress conditions were obtained in USA by Omolayo et al., (2021). This author showed that compared to a good water supply, drought condition significantly decreased plant height, leaf area and number, fresh and total dry mass and net photosynthesis of cowpea genotypes.

The grain production obtained when harvesting the 16 cowpea genotypes taken in our study, presented values between 1,413.5-3,065.5 kg/ha, with an average of 2,207.2 kg/ha (Table 6). The statistical analysis of the obtained results highlighted to *27/2*, *27-B-3a*, *32-B-3*, *25-A1-3a*, *25-A2-4* cowpea genotypes, which recorded significant distinctly production differences, between 1,001.6 and 1,620.6 kg/ha, compared to the control variety (*Jiana*).

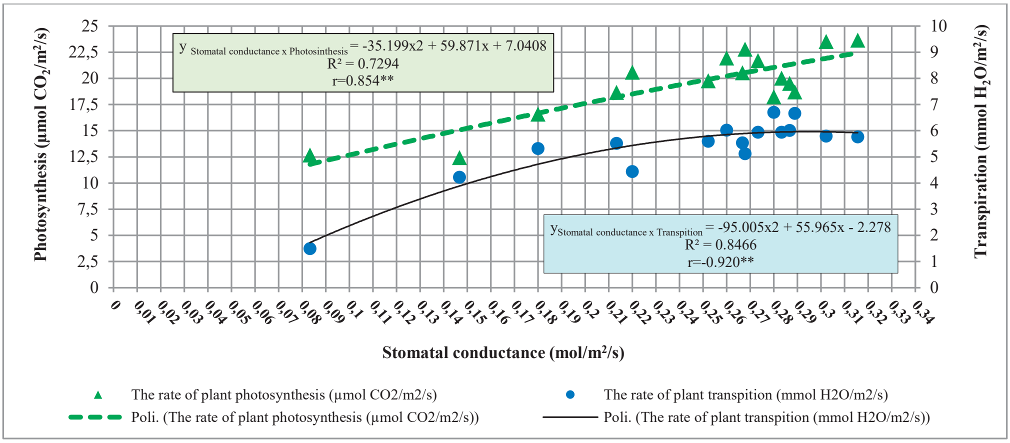

Figure 2. Correlations between stomatal conductance and the physiological processes of plant photosynthesis and transpiration in 16 cowpea genotypes

The results presented graphically in Figure 3 express the functional link between the number of pods and the cowpea production obtained, highlighting a distinctly significant positive correlation (r = 0.703**).

Similar research was conducted in South Africa by Gerrano et al. (2022) and the results obtained show that the number of pods/plant is a major agronomic trait influencing the production potential of cowpea.

Table 6. Production results obtained with the cowpea genotypes tested under the conditions of the sandy soils in the south of Oltenia

No. crt.	Cowpe genotypes	Grain Yield		Difference from the control	
		kg/ha	%	kg/ha	Significance
1	Jiana	1,444.9	100	Control	Control
2	31-E2-2a	2,327.4	161.1	882.5	-
3	27/2	2,928.6	202.7	1,483.7	**
4	27-B-3a	3,065.5	212.2	1,620.6	**
5	32-B-3a	2,446.5	169.3	1,001.6	*
6	27-A4	2,325.4	160.9	880.5	-
7	25-A1-3	2,954.4	204.5	1,509.5	**
8	25-A2-4	2,458.7	170.2	1,013.8	*
9	25-B1-3	1,992.1	137.9	547.2	-
10	25-D1-4	1,579.4	109.3	134.5	-
11	25-D1-5	2,335.4	161.6	890.5	-
12	China T1	2,008.8	139.0	563.9	-
13	China T2	1,413.5	97.8	-31.4	-
14	China T3	1,873.1	129.6	428.2	-
15	China T4	2,244.1	155.3	799.2	-
16	China T5	1,916.7	132.7	471.8	-
		LSD 5%		913.4	
		LSD 1%		1,265.0	
		LSD 0.1%		1,745.2	

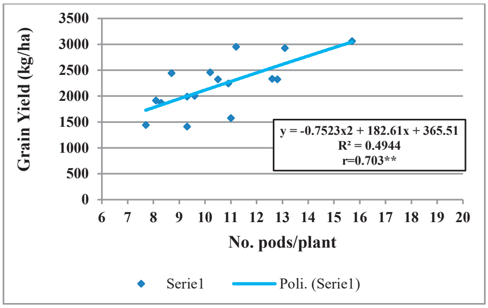

Figure 3. Correlation between number of pods/plant and grain yield obtained in 16 cowpea genotypes

The protein deficit in less developed areas, as a result of the rapid growth of the human population and the demand for animal protein, results in the use of legumes, including cowpea, as a source of protein in traditional grain-based diets. Cowpea seeds, leaves (fresh and dry) and green pods provide high levels of protein, carbohydrates, lipids, vitamins, dietary fiber, minerals, polyunsaturated fatty acids and other nutrients (Belete & Mulugeta, 2022).

Cowpea bean quality analysis revealed different values of biochemical components, depending on the genotype (Figure 4). Thus, the crude protein content of the grain was between 19.3% in the *China T2* genotype and 22.55% in the *25-D1-5* genotype, with an average of the genotypes of 20.97%. The fat content showed the highest value in the *Jiana* genotype (4.85%), and the minimum of 2.7% in the *27-A4* genotype. Similar results were obtained in Nigeria, on 1,541 lines from cowpea germplasm, the percentage of proteins being differentiated in the range of 16-31% (Boukar et al., 2011).

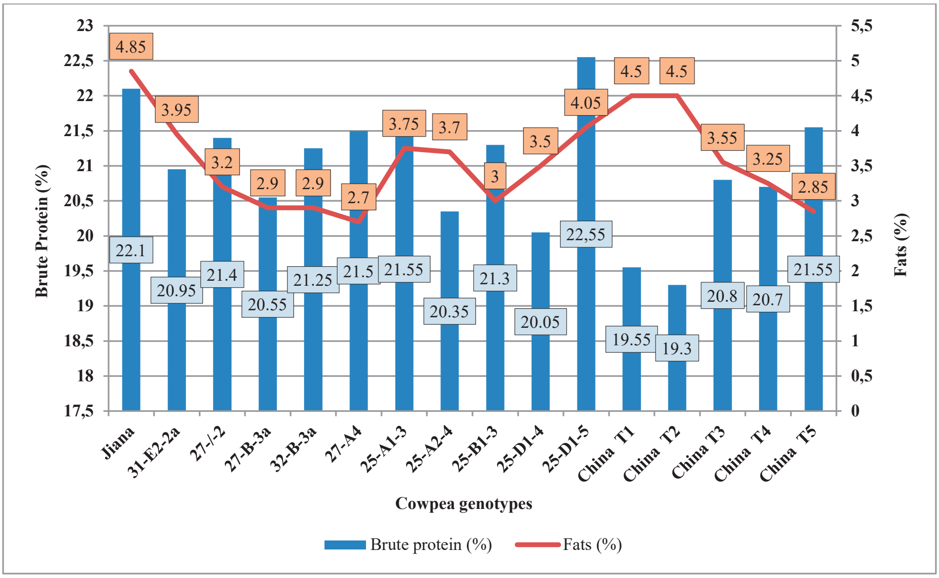

Figure 4. Grain production quality recorded in some cowpea genotypes

CONCLUSIONS

In the conditions of the sandy soils in Romania, the vegetation period of the 16 cowpea genotypes took place during 82-97 days, with a thermal requirement of 1,858.8-2,241.4^{0}C, depending on the genotype.

The results regarding the physiology of the cowpea plant revealed a diurnal variation of the physiological processes of photosynthesis, transpiration and stomatal conductance, closely related to changes in environmental factors, especially solar radiation and air temperature.

In the conditions of the sandy soils in the south of Oltenia, the cowpea production obtained varied between 1,413.5 and 3,065.5 kg/ha, depending on the genotype.

The following genotypes were highlighted: *27/2, 27-B-3a, 32-B-3, 25-A1-3a, 25-A2-4,* which recorded production differences of 1001.6-1620.6 kg/ha compared to the control variety (*Jiana*), statistically assured as significantly and distinctly significantly positive.

Between the number of pods and the grain yield obtained in cowpea genotypes, a distinctly significant positive correlation was revealed (r = 0.703**).

The crude protein content of the cowpea bean showed values between 19.3 and 22.55%, depending on the genotype.

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PARTIAL RESULTS CONCERNING THE INFLUENCE OF THE SEED RATE AND FOLIAR FERTILIZATION ON THE BEHAVIOR OF SOME MILLET GENOTYPES IN THE PEDOCLIMATE CONDITIONS OF S.C.D.A. SECUIENI

Andreea ENEA[1], Teodor ROBU[2], Simona-Florina ISTICIOAIA[1], Alexandra LEONTE[1], Paula-Lucelia PINTILIE[1]

[1]Agricultural Research and Development Station Secuieni, 371 Principala Street, Secuieni, Neamt, Romania
[2]Iasi University of Life Sciences "Ion Ionescu de la Brad", 3 Mihail Sadoveanu Alley, Iasi, Romania

Corresponding author email: andra29nt@yahoo.com

Abstract

Starting with 2022 at the Secuieni Agricultural Research-Development Station, millet (Panicum miliaceum L.) was introduced for study. In this paper are presented partial results obtained regarding the behavior and yields obtained with two millet genotypes experimented with different rates of seeds and foliar fertilization applied products. The obtained results showed that the millet has a good adaptability to the Secuieni pedoclimatic condition registered in 2022. Thus, the millet average yields was of 3144 kg/ha when it was sown with a seed rate of 10 kg/ha, 3324 kg/ha at a seed rate of 14 kg/ha and 3517 kg/ha when the seed rate was 18 kg/ha. Regarding the foliar fertilization of the millet crop, the yields values, both the highest and the lowest, were recorded for the Marius variety sown with a seed rate of 10 kg/ha and foliar fertilized with the commercial product Terra Sorb (2.0 l/ha) (3687 kg/ha), respectively with the commercial product Albit (0.04 l/ha) (3048 kg/ha).

***Key words**: millet, adaptability, yields, foliar fertilization, seed rate.*

INTRODUCTION

Panicum miliaceum L. is one of the world's first domesticated crops and was cultivated before the spread of rice, maize and wheat (Crawford, 2006; Sage et al., 2011).

Ten thousand years ago, it appeared as a staple food in the semi-arid regions of East Asia and later spread throughout the Eurasian region (Lindquist et al., 2005; Cavers et al, 2016).

In Europe, archaeobotanical finds of millet have previously been reported for Neolithic sites whose occupation began as early as the late 7th millennium B.C. (e.g. in the southern and eastern Balkans), as well as for a number of later sites, before the 2nd millennium B.C.

In Romania, archaeological research shows that, in the Neolithic - a period in which people gave up the nomadic life and became sedentary, moving from the lifestyle of hunter - gatherers to that of shepherds and plant growers - people farmed in today's territory of Romania, wheat (more species than today), barley, oats, rye, millet, along with legumes such as beans, lentils.

Among the C_4 panicoids (subfamily: *Panicoideae*), millet (*P. miliaceum* L.) is known to possess morpho-physiological traits that confer tolerance to abiotic stress and show greater adaptability than other cereal crops in different environmental conditions (Bandyopadhyay et al., 2017; Vetriventhan et al., 2018).

The most popular name of *P. miliacum* L. is "proso millet" comes from the general Pan-Slavic name for millet (Croatian: proso, Serbian: просо).

Millet differs from other cereals (maize, wheat, triticale) being considered a low-input ingredient because it requires low production costs (it can be grown on marginal lands) having special agrotechnical advantages: resistance to drought and pest attack, minimum requirements in regarding fertilization (Amadoubr et al., 2013; Changmei and Dorothy, 2014). Adding to these the short

vegetation period, between 60-100 days (Baltensperger, 2002).
Cereal consumption currently supports approximately 50% of the world's total calorie intake and is largely provided by wheat, rice and maize (Das, 2019). At the same time, climate change accelerates land degradation and desertification, and extreme weather events reduce yields (Habiyaremye, 2017).
Given the current and future scenarios, scientists suggest that an effective strategy could be to replace cereal crops that have high water requirements with others adapted to drought conditions (Ndiku, 2014).

MATERIALS AND METHODS

At the Agricultural Research and Development Station Secuieni, in 2022, the millet experiences placed that the aim to:
- establishing the adaptability of some millet genotypes to the pedoclimatic conditions in Central Moldova;
- establishing the optimal seed rate;
- establishing optimal foliar fertilization.
The trifactorial experiment, of the 2 x 3 x 5 type, was located according to the method of subdivided plots, consisting in:

- Factor A - genotypes;
- Factor B - seed rates;
- Factor C - foliar fertilization.

The distance between rows was 12.5 cm in the sowing of the experiment.
P. miliaceum L., today in the Official List of Cultivated Varieties in Romania, has as its representative only the variety Marius.
The genotypes used in the field experiment consisted of:

- the Marius variety (from N.A.R.D.I. Fundulea);
- the local population Secuieni (here referred as "Secuieni").

For sowing these two varieties, three different seed rates were used:

- 10 kg/ha;
- 14 kg/ha;
- 18 kg/ha.

During the vegetation period, foliar fertilization with commercial products was applied to the tested variants:

- Terra Sorb (2.0 l/ha);
- Asfac (1.0 l/ha);
- Albit (0.04 l/ha, respectively 0.06 l/ha).

The experience was placed in the experimental fields of A.R.D.S. Secuieni characterized by a soil of cambic phaeosium type, weakly acid (pH = 6.14), with a low content in humus (2.3%), poorly fertile, little supplied with nitrogen (0.134%), but with a considerable content of phosphorus (77 ppm) and potassium (221 mg/kg) in forms accessible to plants (Trotuș E. et al., 2022).
The millet crop was sown in the optimal epoch for this geographic area, the beginning of May, in a dry land as a result of rainfall deficit recorded in 2022.
The crop was harvested in mid-August, the vegetation period for millet under the conditions of A.R.D.S. Secuieni was 95 days.
The obtained data were processed and statistically interpreted according to the variation analysis method (Jităreanu G., 1994).

RESULTS AND DISCUSSIONS

From a climatic point of view, the 2021-2022 year was characterized as hot and very dry, with the trend being aridification (Figures 1 and 2).

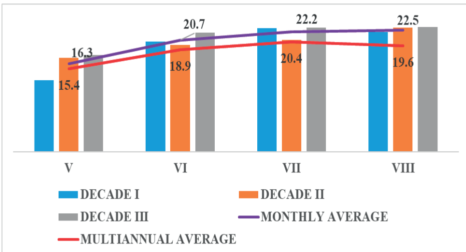

Figure 1. Average temperatures during the millet vegetation period, A.R.D.S. Secuieni, 2022

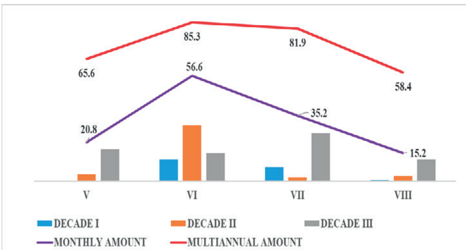

Figure 2. Average precipitation during the millet vegetation period, A.R.D.S Secuieni, 2022

The average temperatures recorded at the meteorological station of A.R.D.S. Secuieni during the millet vegetation period (May-August, 20.5ºC) had a deviation of +1.9ºC compared to the multiannual average from the last 60 years of the same period of the year (18.6ºC).

The temperature difference recorded in May, the month when the millet crop was sown, was 0.9ºC (Figure 1).

June and July, critical months for most agricultural crops, had average temperatures 1.8ºC higher than the multi-year average (Figure 1).

In August, when the millet crop was harvested, the average temperature was 2.9 Celsius degrees higher than the multiannual average (Figure 1).

Regarding the rainfall regime, in the period from the sowing of the millet crop to its physiological maturity, 127.8 mm were unevenly distributed, comparing this value with the multi-year average specific to the same period, of 291.2 mm we note that the deviation has a value of -163.4 mm.

From Figure 2 it can be seen that in the months of May and July, the precipitation had values 3 times lower than the multi-annual average.

In August, when the millet crop was also harvested, the rainfall was approximately 4 times lower than the multi-annual average (Figure 2).

The yield stability in field crops is given by the varieties with a high yield potential, with a superior yield quality and a better adaptability to the biotic and abiotic environmental conditions.

The experimented variants had as a control the local Secuieni population, sown with a seed rate of 14 kg/ha, in an unfertilized system (Table 1).

The results obtained varied from one variant to another.

The unfertilized variants had yields values between 2342 kg/ha (Marius variety sown with rate seed of 10 kg/ha) and 3453 kg/ha (Secuieni sown with rate seed of 18 kg/ha), which they varied depending on the genotype and seed rate.

The yields obtained from the interaction between the three studied factors, genotype x seed rate x foliar fertilization indicated as the optimal variant the interaction between the Marius variety, sown with a seed rate of 10 kg/ha and to which a foliar fertilization with the commercial product was applied Terra Sorb (2.0 l/ha), the yield obtained being 3687 kg/ha (Table 1).

Table 1. Variants tested and yield obtained

Factor A	Factor B (kg/ha)	Factor C (product)	Dozes (l/ha)	Yield kg/ha	Rel. yield %	Dif. kg/ha	Semn
Marius	10	Unfertiliz		2342	76	-745	ooo
		Terra sorb	2.0 l/ha	3687	119	600	**
		Asfac	1.0 l/ha	3677	119	590	**
		Albit 1	0.04 l/ha	3048	99	-39	
		Albit 2	0.06 l/ha	3316	107	229	
	14	Unfertiliz		3337	108	250	
		Terra Sorb	2.0 l/ha	3438	111	351	*
		Asfac	1.0 l/ha	3447	112	360	*
		Albit 1	0.04 l/ha	3480	113	393	*
		Albit 2	0.06 l/ha	3394	110	307	
	18	Unfertiliz		3279	106	192	
		Terra Sorb	2.0 l/ha	3523	114	436	*
		Asfac	1.0 l/ha	3637	118	550	**
		Albit 1	0.04 l/ha	3584	116	497	**
		Albit 2	0.06 l/ha	3623	117	536	**
Secuieni	10	Unfertiliz		2581	84	-506	oo
		Terra Sorb	2.0 l/ha	3366	109	279	
		Asfac	1.0 l/ha	3165	103	78	
		Albit 1	0.04 l/ha	3134	102	47	
		Albit 2	0.06 l/ha	3122	101	35	
	14	Unfertiliz		3087	100	CT	
		Terra Sorb	2.0 l/ha	3253	105	166	
		Asfac	1.0 l/ha	3300	107	213	
		Albit 1	0.04 l/ha	3246	105	159	
		Albit 2	0.06 l/ha	3257	106	170	
	18	Unfertiliz		3453	112	366	*
		Terra Sorb	2.0 l/ha	3487	113	400	*
		Asfac	1.0 l/ha	3513	114	426	*
		Albit 1	0.04 l/ha	3527	114	440	*
		Albit 2	0.06 l/ha	3547	115	460	**
LSD (kg/ha)	5 %			331			
	1 %			456			
	0.1 %			645			

The lowest yield was obtained with the variant sown with the same variety, the same seed rate, but in an unfertilized system, of 2342 kg/ha.

The yield difference between these two variants is 1345 kg/ha (Figure 3).

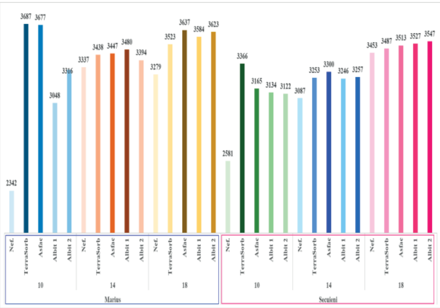

Figure 3. Yields dynamics according to the fertilizer used

Compared to the experience control, the yields differences obtained were ensured and statistically interpreted as follows:

-significant yields increases were obtained at interaction between:

- Marius variety x 14 kg/ha x Terra Sorb (2.0 l/ha) - with a yield increase of 351 kg/ha;
- Marius variety x 14 kg/ha x Asfac (1.0 l/ha) - with a yield increase of 360 kg/ha;
- Marius variety x 14 kg /ha x Albit (0.04 l/ha) - with a yield increase of 393 kg/ha;
- Marius variety x 18 kg/ha x Terra Sorb (2.0 l/ha) - with a yield increase of 307 kg/ha;
- Secuieni population x 18 kg/ha x unfertilized - with a yield increase of 366 kg/ha;
- Secuieni population x 18 kg/ha x Terra Sorb (2.0 l/ha) - with a yield increase of 400 kg/ha;
- Secuieni population x 18 kg/ha x Asfac (1.0 l/ha) - with a yield increase of 426 kg/ha;
- Secuieni population x 18 kg/ha x Albit (0.04 l/ha) - with a yield increase of 440 kg/ha;

-significant yields increases were obtained at interaction between:

- Marius variety x 10 kg/ha x Terra Sorb (2.0 l/ha) - with a yield increase of 600 kg/ha;
- Marius variety x 10 kg/ha x Asfac (1.0 l/ha) - with a yield increase of 590 kg/ha;
- Marius variety x 18 kg/ha x Asfac (1.0 l/ha) with a yield increase of 550 kg/ha;
- Marius variety x 18 kg/ha x Albit (0.04 l/ha) - with a yield increase of 497 kg/ha;
- Marius variety x 18 kg/ha x Albit (0.06 l/ha) - with a yield increase of 497 kg/ha;
- Secuieni population x 18 kg/ha x Albit (0.06 l/ha) - with a yield increase of 460 kg/ha;

- a distinctly significant negative difference was obtained in the variant sown with Secuieni x 10 kg/ha x unfertilized - with a yield difference of - 506 kg/ha;

- very significant negative difference was obtained in the variant sown with Marius variety x 10 kg/ha x unfertilized - with a yield difference of - 745 kg/ha.

A good reaction to foliar fertilization was observed at the tested variants, the lowest yield values being thus recorded in the unfertilized variants, namely:

- average yield for Secuieni population - 3040 kg/ha, with variations between 2581 kg/ha (10 kg/ha rate seed and 3453 kg/ha (18 kg/ha rate seed) (Figure 4);
- average yield for Marius variety - 2986 kg/ha, with variations between 2342 kg/ha (10 kg/ha rate seed) and 3337 kg/ha (14 kg/ha rate seed) (Figure 4).

Figure 4. Yield dynamics for unfertilized variants

For the variants foliarly fertilized with the Terra Sorb (2.0 l/ha) commercial product, the yields had maximum values:

- 3687 kg/ ha - Marius variety sown with 10 kg/ha rate seed (Figure 5);
- 3487 kg/ha - Secuieni population sown with 18 kg/ha rate seed (Figure 5).

Figure 5. Yield dynamics for variants foliarly fertilized with Terra Sorb (2.0 l/ha)

Following the foliar application of the commercial product Asfac, in a dose of

1.0 l/ha, the highest yield was obtained on the following variants, where yields of:

- 3677 kg/ha - Marius variety sown with 10 kg/ha rate seed (Figure 6);
- 3513 kg/ha - Secuieni population sown with 18 kg/ha rate seed (Figure 6).

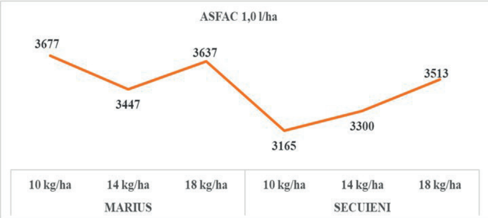

Figure 6. Yield dynamics for variants foliarly fertilized with Asfac (1.0 l/ha)

The application of the commercial product Albit in a dose of 0.04 l/ha generated maximum productions of:

- 3584 kg/ha - Marius variety sown with 18 kg/ha (Figure 7);
- 3527 kg/ha - Secuieni population sown with 18 kg/ha (Figure 7).

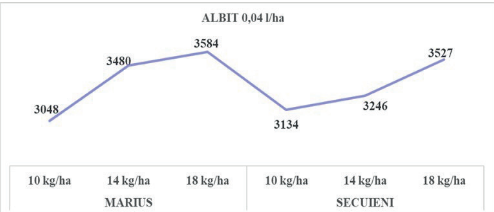

Figure 7. Yield dynamics for variants foliarly fertilized with Albit (0.04 l/ha)

The application of the commercial product Albit in a dose of 0.06 l/ha generated maximum productions of:

- 3623 kg/ha - Marius variety sown with 18 kg/ha (Figure 8);
- 3547 kg/ha - Secuieni population sown with 18 kg/ha (Figure 8).

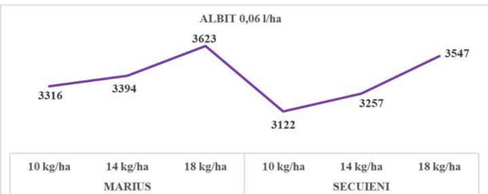

Figure 8. Yield dynamics for variants foliarly fertilized with Albit (0.06 l/ha)

Increasing the dose of Albit (from 0.04 to 0.06 l/ha) generated yields increases of:

- 268 kg/ha - for Marius variety sown with 10 kg/ha;
- 86 kg/ha - for Marius variety sown with 14 kg/ha;
- 39 kg/ha - for Marius variety sown with 18 kg/ha;
- 11 kg/ha - for Secuieni population sown with 14 kg/ha;
- 20 kg/ha - for Secuieni population sown with 18 kg/ha.

CONCLUSIONS

From a climatic point of view, the 2021-2022 year was characterized as hot and very dry.
The tested millet genotypes adapted well to the culture conditions at A.R.D.S. Secuieni.
The yields obtained were between 2342 and 3687 kg/ha.
Yields varied from one technological parameter to another.
At the control variant (interaction: Secuieni population x 14 kg/ha x unfertilized) a yields value of 3087 kg/ha was obtained.
The highest yields value was obtained at the interaction between Marius variety x 10 kg/ha x Terra Sorb (2.0 l/ha) of 3687 kg/ha.
The lowest yields value was obtained at the interaction between Marius x 10 kg/ha x unfertilized of 2342 kg/ha.
For unfertilized variants the highest yields were obtained at the sowing rates of 14 and 18 kg/ha.
The products Terra Sorb and Asfac, administered foliar have a positive influence on millet yields.
Increasing the dose of Albit (from 0.04 to 0.06 l/ha) did not generate significant increases in grain production in the millet culture. This increase not being economically justified.

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OIL CONTENT OF SATURATED AND UNSATURATED FATTY ACIDS IN TRIBENURON-METHYL RESISTANT SUNFLOWER HYBRIDS, DEPENDING ON THE AMOUNT OF MACRONUTRIENTS IN THE SOIL

Adelina GARAPOVA, Hristofor KIRCHEV

Agricultural University of Plovdiv, 12 Mendeleev Blvd, Plovdiv, Bulgaria

Corresponding author email: adelina_garapova@abv.bg

Abstract

In the experimental field of the Department of Crop Science at the Agricultural University - Plovdiv for three years, a field experiment has been conducted. The experiment has been carried out by the method of split-plots in four replications after the predecessor triticale. Five tribenuron-methyl resistant sunflower hybrids (main plots) have been studied: P64LE25 (standard); LG 59.580 SX; Subaro HTS; ES Arcadia SU and Magma SU. The effect of two soil nutrition regimes - lower and higher has been investigated (split-plots). The oil content of the seeds has been studied. The fatty acid composition of the oil has been investigated. It has been found that the hybrids can be arranged in the following descending order regarding the average oil content of the seeds: P64LE25> Subaru> LG 59.580> Magma> Arcadia. The hybrids contain an average of 15% saturated and 85% unsaturated fatty acids. The lowest content of saturated and the highest content of unsaturated fatty acids have been found in hybrid Magma, and the highest content of saturated and lowest content of unsaturated - in hybrid Subaru.

Key words*: sunflower, tribenuron-methyl resistant, saturated and unsaturated fatty acids.*

INTRODUCTION

One of the directions of use of oilseed crops in recent years is for the production of biodiesel, as sunflower is one of the main crops for the countries of the southern part of Europe (Kalligeros et al., 2003; Stoev and Tahsin, 2012).

The seeds of modern sunflower varieties and hybrids contain mainly 40-50% oil (50-60% oil in the nut) and 15-22% protein (Schuster et al., 1980; Yankov, 2009; Lobão et al., 2017; Romanic et al., 2018; Drumeva & Yankov, 2018; Ahmadian et al., 2019; Kanwal et al., 2019; Naila et al., 2019).

Numerous experiments under different soil and climatic conditions prove the primary importance of air temperature in the synthesis of reserve substances (oil, protein, fatty acid composition of the oil). When tested under controlled conditions, it was reliably found that as the temperature increases, the oil content of the seeds decreases and the percentage of protein in the nut increases (Champolivier & Merrien, 1996; Caliskan et al., 2002; Thomaz et al., 2012).

The oil yield is the result of the yield of seeds and seed oil content. That is why many researchers analyze the factors that influence the formation of yield seeds and their components as a starting point for the formation of the final productivity of the crop-oil yield (Palijo et al., 2020; Laskhman et al., 2020; Siahbidi et al., 2020).

Merrien & Champolivier (1995) consider that the fatty acid composition of the oil largely depends on the region of sunflower cultivation, the conditions of the year, nitrogen fertilization and the moisture supply of the crop

MATERIALS AND METHODS

In the experimental field of the Department of Crop Science at the Agricultural University-Plovdiv during the three harvest years - 2018, 2019 and 2020 a field experiment has been conducted. The experiment has been carried out by the method of split-plots in four replications after the predecessor triticale. The effect of two soil nutrition regimes (NR) - lower (NRL) and higher (NRH) has been investigated (main plots). The differences in the content of macronutrients in the soil are a consequence of previous fertilizer experiments conducted on the triticale predecessor (Georgieva, 2019). Five sunflower hybrids, all of the Tribenuron-methyl resistant hybrids group have been studied: P64LE25 - Pioneer® (standard); LG 59.580 SX - Limagrain®; Subaro HTS - Syngenta®; ES Arcadia SU - Euralis®; Magma SU - Caussade semences®. Fat content and fatty acid composition were calculated by gas chromatography (ISO 12966-2:2017).

RESULTS AND DISCUSSIONS

In addition to the oil content in the seeds, the main quality indicator of sunflowers is the composition of the oil. One of the main valuable qualities of sunflower oil is a higher amount of unsaturated fatty acids in the direction of the saturated (Figure 1).

In the first year of the study, the saturated fatty acid content decreased with the higher soil nutritional regimes compared to the lower ones. In the P64LE25 (standard), the content of saturated fatty acids decreased under the influence of a better soil nutrition regime from 11.1% to 10.2%; at LG 59.580 SX - from 10% to 8.4%; at Subaru from 12% to 10.4%; at Magma - from 8.4 to 7.7%. An exception is the Arcadia hybrid, in which the content of saturated fatty acids is lower (8.5%) than in the higher soil fertility (10.5%). The content of unsaturated fatty acids in the current year represents between 88-92.3% of the total composition of the oil in the tested sunflower hybrids.

In the P64LE25 standard, the content of unsaturated fatty acids in the lower soil nutritional regime is 88.9% (48.3% monounsaturated + 40.6% polyunsaturated). The higher soil regime leads to an increase in the content of unsaturated fatty acids up to 89.8% (55.2% monounsaturated + 34.6% polyunsaturated).

In the LG 59.580 SX hybrid, the content of unsaturated fatty acids at the lower nutritional regime is 90% (37.1% monounsaturated + 52.9% polyunsaturated). The higher soil regime leads to an increase in the content of unsaturated fatty acids up to 91.6% (48.2% monounsaturated + 43.4% polyunsaturated).

In the Subaru hybrid, the content of unsaturated fatty acids at the lower nutritional regime is 88% (61.2% monounsaturated + 26.8% polyunsaturated). The higher soil regime leads to an increase in the content of unsaturated fatty acids up to 89.6% (60.6% monounsaturated + 29% polyunsaturated).

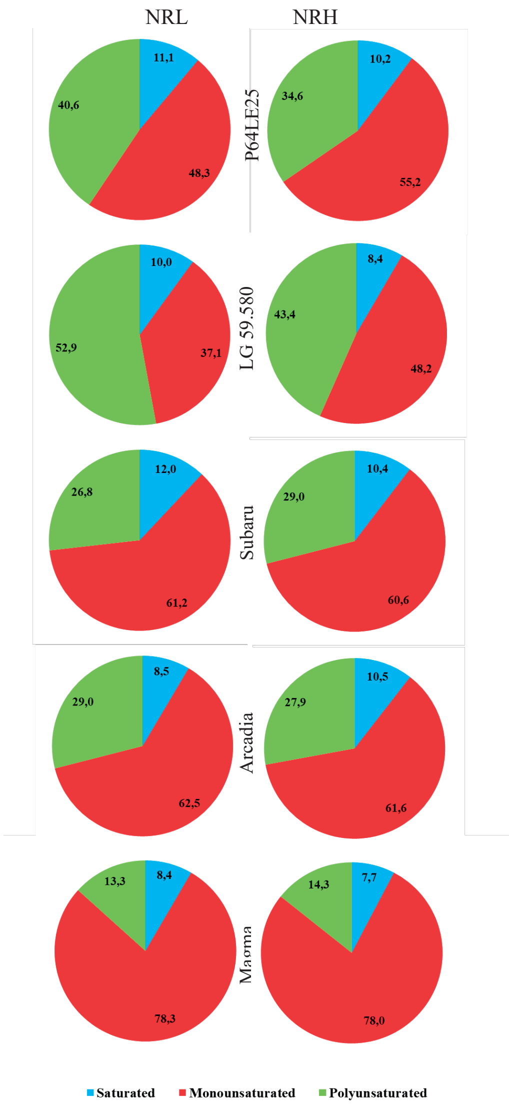

Figure 1. Ratio between saturated and unsaturated (polyunsaturated + monounsaturated) fatty acids, 2018 year

In Magma hybrid, the content of unsaturated fatty acids in the lower nutritional regime is 91.6% (78.3% monounsaturated + 13.3% polyunsaturated). The higher soil regime leads to an increase in the content of unsaturated fatty acids up to 92.3% (78% monounsaturated + 14.3% polyunsaturated).

In Arcadia hybrid, the content of unsaturated fatty acids in the lower nutritional regime is 91.5% (62.5% monounsaturated + 29% polyunsaturated). Only in this hybrid, in the higher soil regime, the content of unsaturated fatty acids decreases to 89.5% (61.6% monounsaturated + 27.9% polyunsaturated).

In the 2019 year of study (Figure 2), the content of saturated fatty acids, similar to the first year, decreased in the higher soil nutritional regime compared to the lower.

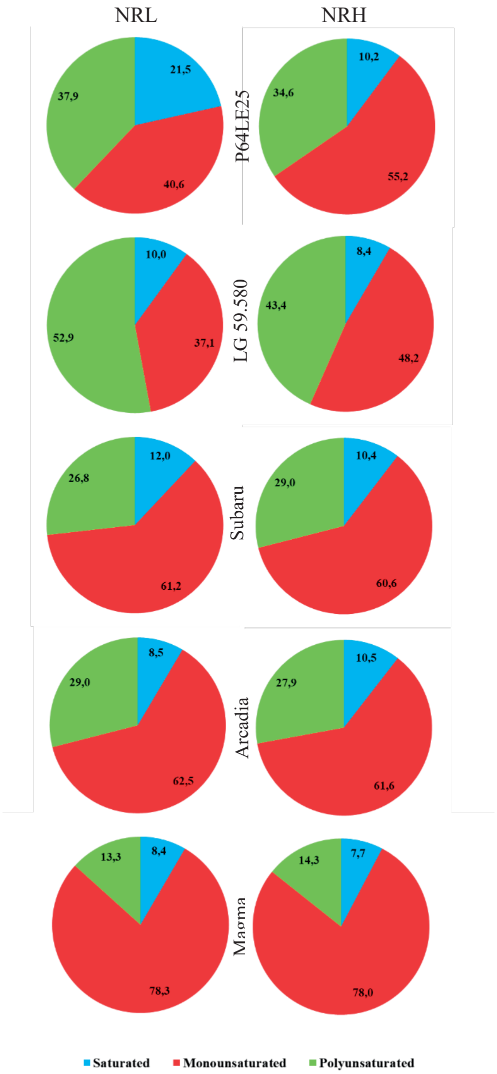

Figure 2. Ratio between saturated and unsaturated (polyunsaturated + monounsaturated) fatty acids, 2019 year

The content of saturated fatty acids decreases in the better soil nutritional regime of the soil as follows: in hybrid P64LE25 (standard) - from 21.5% to 14.3%, in LG 59.580 SX - from 16.5% to 14% and in Subaru from 25.8% to 20.6%.

Exceptions are Magma and Arcadia hybrids, in which, with the higher soil stock with macronutrients, the content of fatty acids increases, respectively from 13.7% to 15.5% for hybrid Magma and from 9.9% to 14.4 % for hybrid Arcadia.

In the second year of the study, the content of unsaturated fatty acids varied between 75.1% and 90.1% of the total oil composition in all tested hybrids.

In the P64LE25 hybrid (standard), the content of unsaturated fatty acids at the lower soil regime is 78.5% (40.6% monounsaturated + 37.9% polyunsaturated). The higher soil nutritional regime leads to an increase in the content of unsaturated fatty acids up to 85.7% (38.2% monounsaturated + 47.5% polyunsaturated).

In the LG 59.580 SX hybrid, the content of unsaturated fatty acids at the lower soil nutritional regime is 83.5% (43.5% monounsaturated + 40% polyunsaturated). In the higher soil regime, the content of unsaturated fatty acids increases to 86% (35.8% monounsaturated + 50.2% polyunsaturated).

In the Subaru hybrid, the content of unsaturated fatty acids in the lower nutrient supply of the soil is 75.1% (46.4% monounsaturated + 28.7% polyunsaturated). The increase in soil regime again leads to an increase in the content of unsaturated fatty acids to 79.4% (49.7% monounsaturated + 29.7% polyunsaturated).

The Arcadia and Magma hybrids are an exception in the second harvest year, as in them, the content of unsaturated fatty acids is reduced due to the better soil regime.

In the Arcadia hybrid, the content of unsaturated fatty acids at the lower soil nutritional regime is 86.3% (48.5% monounsaturated + 37.8% polyunsaturated). In the higher soil regime, the content of unsaturated fatty acids decreases to 84.5% (44.7% monounsaturated + 39.8% polyunsaturated).

In the Magma hybrid, the content of unsaturated fatty acids at the lower soil nutritional regime is 90.1% (74.8% monounsaturated + 15.3% polyunsaturated). The higher soil regime leads to a decrease in the content of unsaturated fatty acids to 85.6% (71.5% monounsaturated + 14.1% polyunsaturated).

In the third year of the study, the content of saturated fatty acids increased in two of the studied hybrids (P64LE25 and Subaru), with a higher soil nutritional regime compared to the lower one. In the LG 59.580 SX, Arcadia and Magma hybrids, the increase in the nutrient supply of the soil leads to a decrease in the content of saturated acids (Figure 3).

In P64LE25 (standard), the content of saturated fatty acids increased in the higher soil nutritional regime compared to the lower one by 0.1%, and in the hybrid Subaru by 2.6%.

In the LG 59.580 SX hybrid, the content of saturated fatty acids decreased under the influence of better soil nutritional regime from 29.3% to 24.9%, in Arcadia - from 16.5% to 14.8% and in Magma - from 11.8% to 11%.

The content of unsaturated fatty acids in this third year of the study was between 70.7 and 89% of the total oil composition for the five studied sunflower hybrids. Increasing the nutrient in the soil affects differently the content of unsaturated fatty acids (Figure 3).

In the P64LE25 hybrid (standard), the content of unsaturated fatty acids at the lower soil nutritional regime is 80.8% (14.8% polyunsaturated + 66% monounsaturated).

The reason for this is probably the climatic conditions in the third year (Garapova, 2021) related to the moisture deficit during seed formation and ripening. This is also confirmed by the studies of Petcu et al., (2001) and EL Sabagh et al., (2019), according to which, under drought conditions, the content of oleic and palmitic acid increases.

The better soil regime leads to a decrease in the content of unsaturated fatty acids to 80.7% (5.6% polyunsaturated + 75.1% monounsaturated).

In the Subaru hybrid, unsaturated fatty acids represented 76.2% of the total oil composition at the lower soil nutritional regime, of which 5.1% were polyunsaturated and 71.1% were monounsaturated. The higher soil regime leads to a decrease in the content of unsaturated fatty acids to 73.6% (7.8% polyunsaturated + 65.8% monounsaturated).

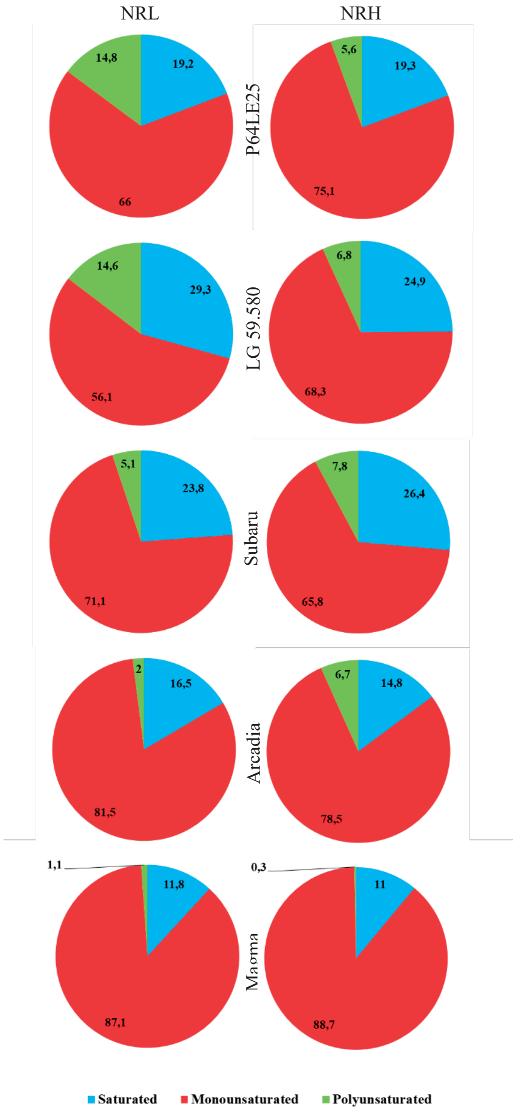

Figure 3. Ratio between saturated and unsaturated (polyunsaturated + monounsaturated) fatty acids, 2020 year

In these two hybrids, the increase in soil fertility harms the content of unsaturated fatty acids, leading to a decrease in their content. In the other three hybrids (LG 59.580 SX, Arcadia and Magma), the better supply of the soil with nutrients leads to an increase in the content of unsaturated fatty acids.

In the LG 59.580 SX hybrid, the content of unsaturated fatty acids at storage 1 is 70.7% (14.6% polyunsaturated + 56.1% monounsaturated). The increase in soil fertility leads to an increase in the content of unsaturated fatty acids to 75.1% (6.8% polyunsaturated + 68.3% monounsaturated).
In the Arcadia hybrid, the content of unsaturated fatty acids at first storage is 83.5% (2% polyunsaturated + 81.5% monounsaturated), and higher soil fertility leads to an increase in the content of unsaturated fatty acids to 85.2% (6.7% polyunsaturated + 78.5% monounsaturated).
In the Magma hybrid, the content of unsaturated fatty acids in the lower soil nutritional regime is 88.2% (1.1% polyunsaturated + 87.1% monounsaturated). In higher soil regimes, the content of unsaturated fatty acids also increases up to 89% (0.3% polyunsaturated + 88.7% monounsaturated).
The average for the three years of the present study, the proportion of essential fatty acids changed differently, depending on the soil nutritional regime (Figure 4).
The content of saturated fatty acid decreased in the higher soil nutritional regime compared to the lower in the three of the studied hybrids (P64LE25, LG 59.580 SX and Subaru). The difference between the two soil regimes in these three hybrids varies between 1.2 and 2.8% in favour of stock 1. In the Arcadia and Magma hybrids, the increase in soil fertility leads to an increase in the amount of saturated fatty acids, respectively by 0.7% in the Arcadia hybrid and by 1% in the Magma hybrid.
The content of unsaturated fatty acids, an average over the three years of the study, represents between 79.8-90% of the total composition of the oil in the studied hybrids. In P64LE25 hybrid (standard), the content of unsaturated fatty acids at the lower soil regime is 82.7% (51.6% monounsaturated + 31.1% polyunsaturated). The higher soil nutritional regime leads to an increase in the content of unsaturated fatty acids to 85.4% (56.2% monounsaturated + 29.2% polyunsaturated).
In the LG 59.580 SX hybrid, the content of unsaturated fatty acids at the first stock is 81.4% (45.6% monounsaturated + 35.8% polyunsaturated). The higher soil regime leads to an increase in the content of unsaturated fatty acids up to 84.3% (50.8% monounsaturated + 33.5% polyunsaturated).

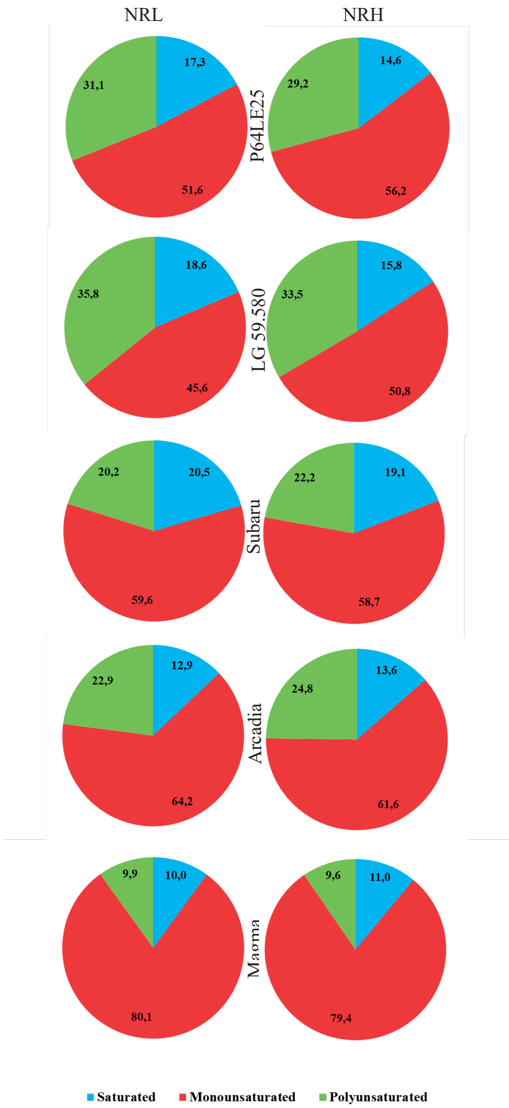

Figure 4. Ratio of saturated to unsaturated (polyunsaturated + monounsaturated) fatty acids, averaged over three years

In the Subaru hybrid, the content of unsaturated fatty acids at the first stock is 79.8% (59.6% monounsaturated + 20.2% polyunsaturated). The higher soil regime leads to an increase in the content of unsaturated fatty acids up to 80.9% (58.7% monounsaturated + 22.2% polyunsaturated).
In the Arcadia hybrid, the content of unsaturated fatty acids in the first soil

nutritional regime is 87.1% (64.2% monounsaturated + 22.9% polyunsaturated). In the Arcadia and Magma hybrid, in the higher soil regime, the content of unsaturated fatty acids decreases to 86.4% (61.6% monounsaturated + 24.8% polyunsaturated).

In the Magma hybrid, the content of unsaturated fatty acids in the first stock is 90% (80.1% monounsaturated + 9.9% polyunsaturated). The higher soil regime leads to a decrease in the content of unsaturated fatty acids to 89% (79.4% monounsaturated + 9.6% polyunsaturated).

CONCLUSIONS

The studied hybrids contain an average of 15% saturated and 85% unsaturated fatty acids. The lowest content of saturated and the highest content of unsaturated fatty acids is found in the Magma hybrid, and the highest content of saturated and lowest content of unsaturated – is in the Subaru hybrid. Soil nutrient content has an ambiguous effect on the ratio of fatty acids in seeds.

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INFLUENCE OF THE SEEDING RATE ON SOME PARAMETERS OF THE GROWTH AND DEVELOPMENT OF *Triticum monococcum* L. IN THE CONDITIONS OF ORGANIC PRODUCTION IN BULGARIA

Tonya GEORGIEVA, Yordan YORDANOV, Malgozhata BEROVA

Agricultural University of Plovdiv, 12 Mendeleev Blvd, Plovdiv, Bulgaria

Corresponding author email: tonia@au-plovdiv.bg

Abstract

The purpose of the study is to establish the growth and development of Triticum monococcum L. in organic farming conditions. The main factors of the study were: year (2018-2019; 2019-2020 and 2020-2021) and seeding rate (500, 700 and 900 germinated seeds/m^2). A three-year field experiment was conducted at the Agricultural University - Plovdiv, using the block method, with a plot of 15 m^2, in 4 repetitions. Phenological development was analysed. Vegetation takes place for 230-233 days. With increasing seeding rate, the number of sprouted plants/m^2 has been proven to increase, reaching up to 506 plants/m^2 at 900 germinated seeds (g.s.)/m^2. The plants develop a height of up to 102.4 cm at a seeding rate of 700 g.s./m^2. With an increase in the seeding rate from 500 to 900 g. s./m^2, the productive tillering decreases from 4.1 to 3.9 number of tillers/plant. The number of productive stems/m^2 varies from 902 (at 500 g.s./m^2) to 968 numbers/m^2 (at 700 g.s./m^2). The year affects all tested factors, as proven - with worse conditions for developing Triticum monococcum L., 2018-2019 stands out.

***Key words**: Triticum monococcum L., growth, phenophases, seeding rate.*

INTRODUCTION

Einkorn (*Triticum monococcum* L.) is a type of wheat that has both wild and domesticated forms. It is assumed that this is the most ancient species and belongs to the group of diploid wheat (2n = 14).

The stability and unpretentiousness of this culture make it possible to obtain acceptable yields from it on weak soils with low agrotechnics in semi-mountainous and mountainous regions. The crop is suitable for scientific research as an alternative food source with the growing interest in organic farming and healthy eating.

Triticum monococcum L. is among the earliest cereals domesticated by man (Feldman, 1976). The abandonment and decline in its production are due (Nesbitt, 1996; Heun et al., 1997) to changes in people's dietary habits, economic conditions and the introduction into production of *Triticum aestivum* L. and *Triticum durum* Desf., which are higher yielding and can easily to be threshed. Interest in this ancient species has also been renewed due to the search for traditional crops suitable for growing in poor and disadvantaged areas, as well as the need to preserve the genetic diversity of field crops (Stagnari et al., 2008; Konvalina et al., 2014; Glamočlija et al., 2015).

Triticum monococcum L. is known to be one of the first cultivated wheat (Salamini et al., 2002). Wheat grains have been found in Fertile Crescent sites. Einkorn has first domesticated in Southwest Asia at approximately 10 500 BC. The exact locality is mentioned by Heun et al. (1997) and Weiss and Zohary (2011), and it is near the Karakadag Mountains in present-day southeastern Turkey. Grains of einkorn have also been found in the region of the Near East. In the years of the Bronze Age, it significantly reduced its area. In Bulgaria, einkorn is a little wider.

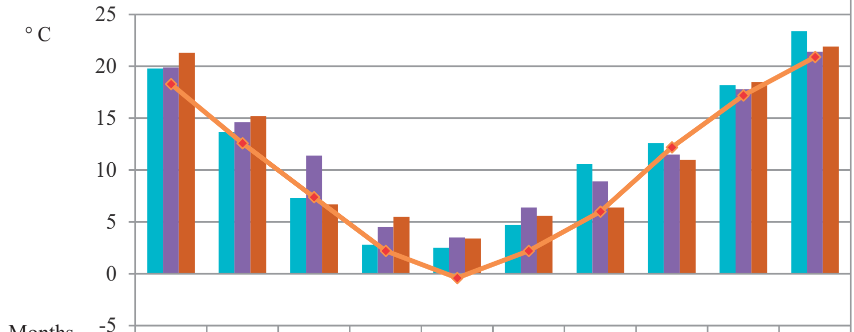
There is a renewed interest in this crop due to the nutritional qualities of the grain, its adaptation to economic cultivation and the high resistance to enemies and diseases, which is an advantage for organic farming. Cultivated einkorn is also a valuable source of genes for the breeding improvement of modern wheat (Zaharieva and Monneveux, 2014).

Sowing density is one of the main agrotechnical factors that have been studied in various places around the world. In Italy, for example, Castagna et al. (1995) studied six different seeding rates (from 100 to 600

germinated seeds (g.s./m^2) in 21 wheat lines. The authors recommend the optimal seeding rate of 300 g.s./m^2, at which they get the highest yield.
In an experiment conducted in Croatia in 2015-2018, the influence of increasing seeding rates (100, 150, 200 and 250 g.s./m^2) on yield and its components were studied (Pospilis et al., 2020). An increase in yield with an increase in seeding rate was found, but differences above 150 g. s./m^2 were not statistically proven.
In southern Italy, Trocoli and Codianni (2005) made a complex study of the influence of the seeding rate on the productivity of three types of wheat, including the einkorn. Unlike emmer and spelt, einkorn realizes the highest yield at the seeding rate at the smallest tested rate - 100 g. s./m^2. However, the authors specify that further studies are needed.
The literature review shows the existence of conflicting and insufficient scientific data related to the influence of the seeding rate on the growth, development and formation of some productive components of the yield. With the present study, we set ourselves the goal - to optimize the seeding rate for local forms of einkorn in the conditions of organic production in Bulgaria.

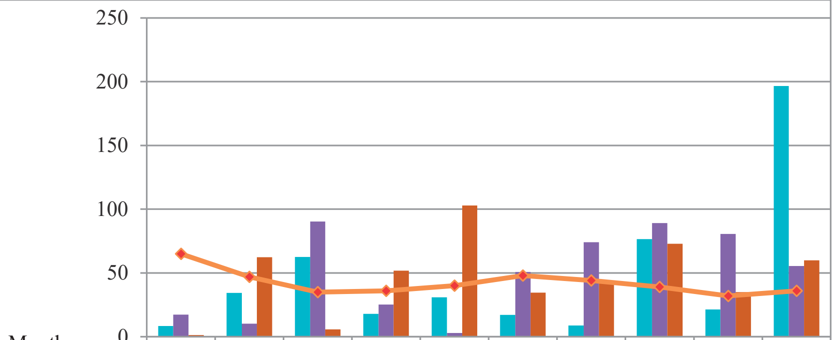
MATERIALS AND METHODS

In 2018-2021, a field experiment was conducted on the field of the first organically certified farm in Bulgaria - the Agroecological Center of the Agricultural University - Plovdiv, which is also a member of the International Federation for Organic Agriculture since 1993.
The seeds of the local form *Triticum monococcum* L. used were biologically produced by the Institute of Plant Genetic Resources "K. Malkov". They are provided with the necessary documents corresponding to Regulation 848/2018.
The experiment is two-factorial; it is laid out in a block method, in four repetitions, after a predecessor common vetch (*Vicia sativa* L. ssp. *sativa*), sown in spring. The main tested factor (A) is the seeding rate with three levels: A1 - 500 germinated seed (g.s.)/m^2, A2 - 700 g.s./m^2 and A3 - 900 g.s./m^2. The reporting area of each plot is 15 m^2. In the statistical processing of the data, the year was also analyzed as a second factor in the three-year experiment.
After harvesting the selected predecessor - spring vetch, a shallow plowing (18-20 cm) and a single disking at a depth of 10-12 cm was carried out. During the monitoring of the crop, no attacks of diseases and enemies were reported, as a result of which plant protection was not required. In early spring, as soon as possible, harrowing is carried out against late-autumn and early-spring weeding in the plots, even when the weeds are in their initial stages and the einkorn is at the end of the tillering phase.
The indicators that are reported are: emergence, phenological development, dynamics of growth in height, dynamics of tillering and productive stems.
Statistical data processing was performed with SPSS V. 9.0 for the Microsoft Windows program, using Duncan's method, Anova.
The agroecological center is located on alluvial-meadow soils (Mollic Fulvisols) (FAO, 1988). Nitrogen availability is weak; it is good with potassium and weak to medium with phosphorus (Popova and Sevov, 2010). The analyzed soil reaction is neutral to slightly alkaline and has a low percentage of organic matter.
In the autumn of the first year (2018), the weather conditions were not particularly favorable for the timely preparation of the soil for sowing (Figure 1). Temperatures in autumn are relatively high, but rainfall is extremely low. Later in the growing season, this deficit is compensated by rainfall peaks in November, April and especially June.
The growing year 2019/2020 is more favorable, which has been taken into account since autumn. The complex agrometeorological conditions ensure normal preparation for sowing and optimal conditions for the phenophases.
In the third experimental year, 2020/2021, conditions were also unfavorable for timely sowing. In October, however, heavy rainfall (62.3 mm/m^2) falls, stimulating rapid germination (Figure 2). By the end of December, the plants enter the tillering phase. In the next phenological phases in the spring, the plants go through the same periods in the three years.



Months	IX	X	XI	XII	I	II	III	IV	V	VI
t C° for 2018-2019	19,8	13,7	7,3	2,8	2,5	4,7	10,6	12,6	18,2	23,4
t C° for 2019-2020	19,9	14,6	11,4	4,5	3,5	6,4	8,9	11,5	17,8	21,4
t C° for 2020-2021	21,3	15,2	6,7	5,5	3,4	5,6	6,4	11	18,5	21,9
t C° Average for the period 1981-2010	18,3	12,6	7,4	2,2	-0,4	2,2	6	12,2	17,2	20,9

Figure 1. Average monthly temperatures (t°C) during the growing season of *Triticum monococcum* L. in 2018/2019, 2019/2020 and 2020/2021



Months	IX	X	XI	XII	I	II	III	IV	V	VI
Precipitation, 2018-2019	8,3	34,3	62,5	17,9	30,9	17,2	8,8	76,5	21,3	196,7
Precipitation, 2019-2020	17,4	10,2	90,4	25,3	3	50,7	74,1	89,1	80,7	55,4
Precipitation, 2020-2021	1,3	62,3	5,7	51,8	103	34,6	42,2	72,9	34,9	60
Precipitation, average for the period 1981-2010	65	47	35	36	40	48	44	39	32	36

Figure 2. Amount of precipitation (mm/m^2) during the growing season of *Triticum monococcum* L. in 2018/2019, 2019/2020 and 2020/2021

RESULTS AND DISCUSSIONS

Germination. The presence of chaff in einkorn seeds affects the speed and degree of germination due to the strength with which they cover them. Ehsanzadeh (1998) spoke in his study about this predetermined genetic specificity of the grain in einkorn and its negative influence on seed germination and subsequent development.

This indicator is also influenced by agro-meteorological conditions, the sowing time, and the seeding rate.

Simultaneous sprouting is extremely important for the good and harmonious development of the crop. Weed control becomes difficult when this wheat is grown organically (without chemical plant protection) and sowing is done in autumn. Therefore, it is very important to ensure the optimal density of sprouted plants. This ensures a better and uniform absorption of nutrients from the soil. Moreover, in winter, it is possible to reduce the sprouted plants to 50%, according to some authors (Jablonskytė-Raščė et al., 2013). They have found that if 320-360 plants per m^2 have sprouted, 45-46% of the plants survive the winter, which significantly reduces density.

Our results also show such a large percentage reduction in germination (Table 1). The data in the table 1 shows that in the first year of the study (2018/2019), the sprouted plants were from 395 to 538 per m^2, which is 59.7% to 79.0% of the seeding rate. Although a greater number of sprouted plants was reported with an increase in the seeding rate, a trend was found for a lower percentage of sprouted plants at the higher seeding rate (900 g.s./m^2).

The established smaller % of sprouted plants at the highest seeding rate was also confirmed in the second and third experimental years.

We can also add that the soil's non-uniform distribution of moisture causes einkorn's non-uniform germination and, accordingly, the different number of sprouted plants.

Table 1. Influence of seeding rate on the number of sprouted plants/m^2 of *Triticum monococcum* L., by years

Variant	2018/2019	2019/2020	2020/2021
500 g.s./m^2	395 c	335 b	366 c
700 g.s./m^2	445 b	458 a	455 b
900 g.s./m^2	538 a	470 a	508 a

*Means followed by the same letter are not statistically different (P<0.05) by Duncan's multiple range test

When testing the independent influence of the year factor on average over the three-year study period, it was found that the most favorable conditions for germination were in 2018. In 2019, plants germinated with a proven smaller number per m^2, followed by 2020. Regarding the influence of the seeding rate, it is found that with an increase in the seeding rate from 500 to 900 g.s./m^2, the number of sprouted plants per m^2 is proven to increase. We can summarize that by increasing the seeding rate by up to 80% (from 500 to 900 g.s./m^2), the number of sprouted plants increases by up to 38.4%.

Table 2. Influence of year and seeding rate on the number of sprouted plants/m^2 of *Titicum monococcum* L., average for the period

Influence of factor Year			Influence of factor Seeding rate		
Variant	Number/ m^2	%	Variant	Number/ m^2	%
2018-2019	459.3 a	100.0	500 g.s./m^2	365.3 c	100.0
2019-2020	421.1 b	91.7	700 g.s./m^2	452.8 b	124.0
2020-2021	443.2 a	96.5	900 g.s./m^2	505.6 a	138.4

*Means followed by the same letter are not statistically different (P<0.05) by Duncan's multiple range test

Phenological development. Despite the difficult and slightly delayed tillage of the soil, sowing in all three years was carried out in October, which for the conditions of Bulgaria, is perceived as an optimal time (Table 3). The plants germinate quite irregularly in the period 5-10 November. The reason is the need for sufficient moisture and its uneven distribution in the soil. This negative effect on germinating was commented on in a publication by other Bulgarian authors, too (Stamatov et al., 2017a).

Table 3. Phenological development of *Triticum monococcum* L.

Phenological phases	Phases	Sowing		
		24.10.2018	12.10.2019	27.10.2020
Germination	BBCH 11	8.11.2018	10.11.2019	5.11.2020
Third leaf	BBCH 18	14.12.2018	7.12.2019	15.12.2020
Tillering	BBCH 28	23.12.2018	23.12.2019	22.12.2020
Stem elongation	BBCH 38	25.04.2019	21.04.2020	21.04.2021
Heading	BBCH 59	16.05.2019	15.05.2020	15.05.2021
Milk maturity	BBCH 78	8.06.2019	8.06.2020	7.06.2021
Wax maturity	BBCH 89	19.06.2019	17.06.2020	18.06.2021
Full maturity	BBCH 99	26.06.2019	27.06.2020	26.06.2021

Germination also affects the varying duration of the interphase period until entering the 3rd leaf phase - from 27 (2019) to 40 days (2020). The plants enter approximately the same period in tillering - from December 22 to 23. The subsequent phases, up to ripening, proceed normally in all three years, despite certain differences in agro-meteorological conditions. The growing season ends on June 26-27 for 230 to 233 days. All this shows the great adaptability of the species, which successfully overcomes specific meteorological differences in the experiment period. Our conclusions confirm the results of a study done earlier **by** Zorovski et al. (2018).

Dynamics of tillering. Tillering is a biological specificity in cereal crops, which is highly dependent on the genotype, but can also be controlled by agrotechnical factors. Figure 3 presents the dynamics of tillering of *Triticum monococcum* L. during the three years of the experiment, respectively by phases – tillering, stem elongation and heading.

The tillering phase begins on December 22-23 in experimental years and continues until April 21-25 in subsequent years. Within these months in 2018/2019, the plants form a total of up to 4.5 tillers/plant, which by the end of the growing season are reduced by about 36% - to 2.9 tillers/plant.

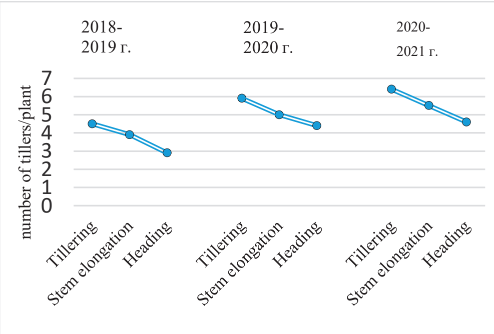


Figure 3. Dynamics of tillering of *Triticum monococcum* L, number of tillers/plant, by phases and by years

In the second experimental year, the maximum number of tillers was much higher - up to 5.9 number/plant, which we explain by the better combination of temperatures and more precipitation in March and April (2020) compared to the first year. Within the further development of the einkorn, the crop self-regulates its density, with only 3.6 productive tillers remaining by the end of the growing season. Thus, 61% of the tillers formed at the beginning of the growing season remain in the crop.

In the third year, the trend is the same. At the end of the growing season, the plants remain with 4.6 productive tillers/plant, 71.9% of the previously formed total number of tillers.

When analyzing the influence of the seeding rate by year, it is found that in the first year, there is no difference between the 500 and 700 g.s./m^2 variants in terms of this indicator (Table 4). As the seeding rate increases to 900 g.s./m^2, the productive tillering decreases. In the third year, the trend is the same - with an increase in the seeding rate, it has been proven that productive tillering decreases. In the second year, the results are mixed. We can summarize that plants form the most productive tillers at 500 g.s./m^2, followed by 700 and 900 g.s./m^2.

Table 4. Influence of seeding rate on the number of productive tillers/plant of *Triticum monococcum* L, at the end of the growing season in., by years

Variant	2018/2019	2019/2020	2020/2021
500 g.s./m^2	3.0 a	4.4 a	4.8 a
700 g.s./m^2	3.0 a	4.3 a	4.6 ab
900 g.s./m^2	2.7 a	4.5 a	4.4 b

*Means followed by the same letter are not statistically different (P<0,05) by Duncan's multiple range test

Table 5. Influence of year and seeding rate on the number of productive tillers/ plant of *Triticum monococcum* L., average for the period

Influence of factor Year			Influence of factor Seeding rate		
Variant	Number/ m^2	%	Variant	Number/ m^2	%
2018-2019	2.9 a	100.0	500 g.s./m^2	4.1 a	100.0
2019-2020	4.4 a	151.7	700 g.s./m^2	4.0 a	97.6
2020-2021	4.6 a	158.6	900 g.s./m^2	3.9 a	95.1

*Means followed by the same letter are not statistically different (P<0.05) by Duncan's multiple range test

The summary analysis of the effect of year and seeding rate for the study period is presented in Table 5. The second and third experimental years provided proven better conditions for tillering than the first year. In the third year, the plants reach a maximum productive tillering of 4.6 productive tillers per plant. With an increase in the seeding rate from 500 to 900 g.s./m^2, productive tillering decreases from 4.1 to 3.9 tillers/plant.

Plant height. Growth in height is an indicator of active growth processes and biomass accumulation. The data in Table 6 shows that the plants reached their highest height in 2020, which we define as the year with the most favorable conditions. Plants reached a height of 112 to 114.8 cm, and no significant differences

were found between the seeding rates tested. The plants remained the lowest in 2019.
The summarized statistical processing for the three years shows a strong influence of the year on this indicator. The differences reach 33.1 to 37.7% and are statistically proven. The seeding rate had a minor effect on plant height and this was not statistically proven (Table 7).

Table 6. Influence of seeding rate on plant height of *Triticum monococcum* L., cm, by years

Variant	2018/2019	2019/2020	2020/2021
500 g.s./m^2	79.9 a	114.8 a	109.4 a
700 g.s./m^2	84.9 a	112.9 a	109.3 a
900 g.s./m^2	81.9 a	112.0 a	109.4 b

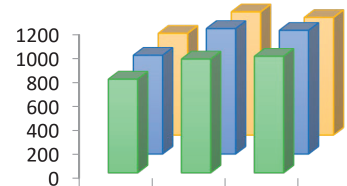
*Means followed by the same letter are not statistically different (P<0.05) by Duncan's multiple range test

Table 7. Influence of year and seeding rate on plant height of *Triticum monococcum* L., cm, average for the period

Influence of factor Year			Influence of factor Seeding rate		
Variant	Plant height, cm	%	Variant	Plant height, cm	%
2018-2019	82.2 b	100.0	500 g.s./m^2	101.4 a	100.0
2019-2020	113.2 a	137.7	700 g.s./m^2	102.4 a	101.0
2020-2021	109.4 a	133.1	900 g.s./m^2	101.1 a	100.0

*Means followed by the same letter are not statistically different (P<0.05) by Duncan's multiple range test

Productive stems. The productive stem is a function of the number of plants germinated and productive tillering. The accumulation of biomass in the crop and the processes of self-regulation in terms of tillering are prerequisites for developing more productive stems. The study of these reactions gives valuable information about the potential of the crop to form a high yield under certain agrometeorological conditions.
Einkorn formed many productive stems even in the unfavourable 2018/2019 year (Figure 4) - 782 to 850 spike-bearing stems, which is most likely due to the higher degree of productive tillering. This year, the tendency to increase the number of productive stems with an increase in the seeding rate to 900 g.s./m^2 is taken into account. In the following two years, the most formed spike-bearing stems were the variants with 700 g.s./m^2.
The summary data for the three years confirms the strong influence of the year. The optimal seeding rate for this indicator is 700 g.s./m^2 (Table 8).



	2018/2019	2019/2020	2020/2021
500 g.s./m2	782	950	973
700 g.s./m2	824	1047	1032
900 g.s./m2	850	1029	982

Figure. 4. Influence of seeding rate on the formation of the productive stems, number/m^2, by years

Table 8. Influence of year and seeding rate on the number of productive stems of *Triticum monococcum* L./m^2, the average for the period

Influence of factor Year			Influence of factor Seeding rate		
Variant	Number/m^2	%	Variant	Number /m^2	%
2018-2019	819 b	100.0	500 g.s./m^2	902 a	100.0
2019-2020	1009 a	123.2	700 g.s./m^2	968 a	107.3
2020-2021	996 a	121.6	900 g.s./m^2	954 a	105.8

*Means followed by the same letter are not statistically different (P<0.05) by Duncan's multiple range test

CONCLUSIONS

When sowing in an optimal period (October) and a seeding rate of 500 to 900 g.s./m^2, 395 to 538 plants germinate, respectively, constituting 79 to 59.7% of the sown seeds. As the seeding rate increases, the germination percentage decreases.
Depending on weather conditions, plants go through germination and third leaf phases at different times. They enter the tillering phase between December 18 and 23 in all three years, regardless of the differences in the duration of the past phases. Despite the specifics in the agrometeorological conditions in the three years, the following phases - stem elongation, heading and ripening, proceed approximately identically. Vegetation ends in 230 to 233 days. For the growing season, einkorn formed 4.4 to 4.6 productive tillers per plant in the more favorable second and third experimental years, 51.7 to 58.6% more than the first year. With an

increase in the seeding rate from 500 to 900 g.s./m^2, the productive tillering decreases by nearly 5%.

The agrometeorological conditions of the year have a proven influence on the height of the plants. In the more favorable second and third years, the plants reached a height of 109.4 and 113.2 cm, which exceeded the values of the first year by 33.1 to 37.7%. The seeding rate does not significantly affect plant height.

The number of formed productive stems reached 1009/m^2 in 2020 and 996 in 2021, 21.6 to 23.2% more than the less favorable year 2019. The most productive stems were formed at a seeding rate of 700 g.s./m^2.

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EFFECTS OF DIFFERENT SULPHUR RATES AND ROW SPACING ON OILSEED RAPE

Lucian-Constantin HARAGA[1, 2], Viorel ION[2], Ioan PUIU[1]

[1]Iasi University of Life Sciences "Ion Ionescu de la Brad", 3 Mihail Sadoveanu Alley, Iasi, 700490, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, 011464, District 1, Bucharest, Romania

Corresponding author email: haragalucian@yahoo.com

Abstract

Oilseed rape is an important crop for the 21st century, its oil being widely used for producing biodiesel, due to its effect on reducing exhaust emissions, as well as in human alimentation. Sulphur is a secondary macronutrient which is important in oilseed rape production as it is interacting strongly with nitrogen having a great impact on seed yield. Row spacing in oilseed rape cultivation is a key element due to its impact on plant architecture, as oilseed rape is a plant with high branching ability, especially in the case of newly created hybrids. Branching can help surpass plant population losses due to environmental factors, it having an important contribution to the seed yield establishment. A study on the interaction between sulphur fertilization rate and row spacing is important to finding the optimum of these elements of oilseed rape production technology, and our research aimed to identify them in the agro-climatic conditions of NE Romania over 3 years, in 2021-2023. Variant with 37.5 cm row spacing and sulphur fertilizer rate of 72 kg SO_3 per hectare generated the highest statistically significant yield of 3,689 kg per hectare compared to the control variant yield of 2,832 kg per hectare. The medium row spacing and high sulphur rate have resulted in higher yields associated with better development of yielding components such as number of branches per plant, number of siliques per plant, and number of seeds per plant, compared to variants with closer row spacing and low sulphur fertilizer rates.

Key words: *oilseed rape, sulphur fertilizer, row spacing, yielding components, seed yield.*

INTRODUCTION

Oilseed rape (*Brassica napus* ssp. *napus* L.) is an important crop for the 21st century, ranking the third place in world vegetable oil production, it being widely used for producing biodiesel, due to its effect on reducing exhaust emissions, as well as in human alimentation especially after development of low erucic acid content hybrids.

In addition to nitrogen (N), sulphur (S) fertilization is considered a critical factor in high yielding oilseed rape crops (Fismes et al., 2000), S deficiencies frequently restricting the yield at this important oil crop (Grant & Bailey, 1993).

Throughout decades of research on the effect of sulphur fertilization it has become evident that, this often-forgotten secondary macronutrient has a substantial impact in oilseed rape crop production.

Sulphur is a constituent of certain amino acids needed for protein synthesis in oilseed rape (Grant & Bailey, 1993; Ngezimana & Agenbag, 2014; Rameeh et al., 2021), such as methionine which is an essential amino acid, as well as cysteine which is a non-essential amino acid, these allowing the formation of disulfide bonds for protein structure and function (Brosnan & Brosnan, 2006). Chemical analysis of seeds obtained in field crops fertilized with high sulphur content showed a significant increase in napin and an important decrease in the level of cruciferin compounds (Poisson et al., 2019). Sulphur is a component of plant amino acids, proteins, vitamins, and enzyme (Varényiová et al., 2017; Ivanov & Harizanova, 2022).

Glucosinolates contain sulphur (Grant et al., 2012), and therefore S fertilization increased the glucosinolates content (Fismes et al., 2000), but their level is still well below the limit set for the canola standard (Hocking et al., 1996). Also, sulphur plays an essential role in the synthesis of chlorophyll and is also an important component for the synthesis of oil (Brennan & Bolland, 2008; Rehmanuh et al., 2013).

Sulphur is more important for reproductive development (grain yield and quality) than vegetative growth (dry matter production) (Ngezimana & Agenbag, 2014).
Sulphur fertilization contributes to improved N-use efficiency (Fismes et al., 2000; Rameeh et al., 2021). Nitrogen and sulphur applied should be balanced so that a limitation of one does not restrict yield response to the other (Malhi et al., 2005). The results of several of the studies focused on the optimization of nutrition of oilseed rape confirm that the highest seed yield was reached at treatments where nitrogen was applied in combination with sulphur (Mansoori, 2012; Jackson, 2000).
Nitrogen and sulphur nutrition during the growth are tightly linked, and their interactions, as reflected by plant uptake, are synergistic at optimum rates and antagonistic at excessive levels of one of the both (Schnug et al., 1993; Fismes et al., 2000; Poisson et al., 2019). Too much N combined with inadequate S can lead to a nutrient imbalance that can restrict protein synthesis and reduce rapeseed growth and seed yield (Grant et al., 2012). Sulphur deficiency mainly leads to a decrease in nitrogen uptake, which will suppress vegetative development of the plant with an important effect on yield components and finally decreasing seed yield (Schnug et al., 1993; Brennan and Bolland, 2008). The requirement of S for optimum seed yield and quality tends to increase with increasing amount of N applied, and should be considered when planning for S fertilization (Malhi et al., 2005). The ratio N:S should be between 5:1 and 7:1 when fertilizer N is applied (Karamanos et al., 2007; Ngezimana & Agenbag, 2014).
Lack of adequate sulphur sources for oilseed rape plants have directly impacted the pathogen resistance mechanism, weakening the plants resistance towards microbial and fungal infections produced by pathogens *Botrytis cinerea* and *Phytophthora brassicae* (Dubuis et al., 2005).
Sulfur deficiency of rapeseed is a major concern in many parts of the world as oilseed rape requires higher levels of S for optimum yield than do most other agronomic crops, and therefore effective S management is an important part of oilseed rape production (Grant et al., 2012).
Row spacing, an important element at seeding time and for plant phenotypical elements developments, has a significant impact on radiation usage efficiency and late stage crop lodging. Also, it is one of the most impactful elements in oilseed rape production technology with effects on plant architecture as it can unleash the plants genetic potential of production through branching.
Many scientists reported that narrow row spacing resulted in maximum seed yield (Hussain et al., 2020). So, research has highlighted that using 15 cm row spacing will increase radiation usage efficiency due to increased competition for light and heat and reduce crop lodging compared to wider row spacing such as 25 cm as plants are able to form a network of resistance between them (Kuai et al., 2021). Plants seeded in 15-cm rows yielded more per area, produced more pods per plant and lodged less than those in 30-cm rows (Morrison at al., 1990). The highest yields were achieved at a density of 45×10^4 plants ha^{-1} in combination with 15 cm row spacing (Kuai et al., 2015). Also, research on row spacing has shown that closer rows at 18 cm spacing will suppress better weed development and thus reduce the number of weeds per surface compared to wider 33 cm row spacing (Kwiatkowski, 2012). Widening of the row-spacing has led to a decrease in the number of pods per plant and the number of plants per unit area and an increase in the number of seeds per pod parameter, while 1000-seed weight was not affected, results that did not confirm a positive effect of sowing oilseed rape in rows wider than 12.5 cm (Krček et al., 2019). Practically, narrow row spacing or higher population of plants are the most helpful ways to regulate the growth of weed species (Hussain et al., 2020).
Advantages of using wider row spacing (33 cm) have been the increased branching of the plant due to excess space between the rows of plants and also an improvement of the yielding element number of siliques formed per plant (Kwiatkowski, 2012). More branching and producing more pods is primarily how oilseed rape responds to the increased space in wider rows, the same response being seen when plant density is low in areas of the stand, winter oilseed rape having a tremendous ability to

compensate by adding branches and pods as space allows (Wysocki, 2020). So, branching can help surpass plant population losses due to environmental factors, it having an important contribution to the seed yield establishment.

A study on the interaction between sulphur fertilization rate and row spacing is important to finding the optimum of these elements of oilseed rape production technology, and our research aimed to identify them in the agro-climatic conditions of NE Romania over 3 years, in 2021-2023.

MATERIALS AND METHODS

Field experiments were conducted over a period of 3 years, respectively in 2020, 2021, and 2022, in the NE Romania (47.5188° N, 27.4490° E), Bivolari commune, Iasi County, in Moldavian plain conditions.

The research was performed under rainfed conditions on a very fertile soil from the class of Chernozems with the top horizon Am-molic. Soil pH is of 7.52, total nitrogen content is of 15.09 mg/kg, phosphorus content is of 21.00 mg/kg, and potassium content is of 228.54 kg/kg.

By comparing the climatic data as historical averages and the averages during 3 years of research, one can observe a consistent pattern of warming especially in the cold months between October and March with an average increase of 3ºC for this period (Table 1).

In terms of annual precipitation, during the 3 years of research, there was registered a deficit of 41.8 mm which shows deteriorating conditions for crop growing, but especially for formation of the oilseed rape yielding component in springtime (Table 2). Lack of sufficient water supply in mid to late plant growth period is a limiting factor for yield as many of the yield components such as number of seeds per silique, number of siliques per plant are all reduced in this water supply situation. During the 3 years of research, the variability of conditions ensures that the field experiments were conducted under different meteorological conditions which increase the general character of the obtained results. It can be observed the increasingly extreme meteorological phenomenon as both annual and monthly averages, dry years with concentrated rains, cooler years with optimum water levels, and long draught periods followed by torrential rains.

Table 1. Monthly temperature in the period 2020-2022 (Bivolari commune, Iasi County, Romania)

Year	Temperature - Monthly Average (°C)												Annual Mean
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	
2020	1.1	4.1	7.2	11.2	14.6	21.9	22.3	23.5	19.6	14.4	5.3	2.4	12.3
2021	0.2	-1.6	3.7	8.6	15.4	20.9	24.1	21.3	15.7	9.9	6.6	0.0	10.4
2022	0.5	3.8	3.7	10.4	16.8	22.1	23.6	23.5	16.1	12.3	5.7	1.0	11.6
Average	0.6	2.1	4.9	10.1	15.6	21.6	23.3	22.8	17.1	12.2	5.9	1.1	11.4
Historical Average	-3.8	-2.0	2.9	10.8	18.9	23.3	24.4	23.5	16.8	9.5	2.8	-1.1	10.5
Difference	4.4	4.1	2.0	-0.7	-3.3	-1.7	-1.1	-0.7	0.3	2.7	3.1	2.2	0.9

Table 2. Monthly precipitation in the period 2020-2022 (Bivolari commune, Iasi County, Romania)

Year	Rainfall - Monthly Average (mm)												Annual Sum
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	
2020	3.4	32.0	15.7	1.8	132.0	98.4	30.4	5.8	26.3	80.1	13.0	41.0	479.9
2021	19.1	28.2	45.4	40.2	67.4	109.4	40.5	132.8	6.6	3.5	10.3	61.2	564.6
2022	9.1	10.8	8.4	52.7	21.5	35.1	28.4	61.3	85.6	13.5	54.4	18.9	399.7
Average	10.5	23.7	23.2	31.6	73.6	81.0	33.1	66.6	39.5	32.4	25.9	40.4	481.4
Historical Average	27.9	25.8	28.4	43.4	55.1	80.7	69.1	55.4	52.3	23.5	30.7	30.9	523.2
Difference	-17.4	-2.1	-5.2	-11.8	18.5	0.3	-36.0	11.2	-12.8	8.9	-4.8	9.5	-41.8

Field research was organized as subdivided experimentation plots with 4 replications and with the following experimental factors:

- Factor A - Sulphur rate, with 4 treatments:
- a1 - 0 kg SO_3/ha;
- a2 - 24 kg SO_3/ha;
- a3 - 48 kg SO_3/ha;
- a4 - 72 kg SO_3/ha.

- Factor B - Row spacing, with 5 treatments:
  - b1 - 12.5 cm;
  - b2 - 25 cm;
  - b3 - 37.5 cm;
  - b4 - 45 cm;
  - b5 - 70 cm.

The variant a1b1 (0 kg/ha sulphur and 12.5 cm row spacing) was taken as control variant.

The oilseed rape hybrid selected for the field experiments was DKC Exception, a widely used hybrid due to its high yield potential, stable production in different year conditions and its potential to use the nitrogen from the soil. The entire experimental field received a basic fertilization of 60 kg N/ha, 60 kg P_2O_5/ha and 60 kg K_2O/ha by applying 400 kg/ha of complex fertilizer 15:15:15 before soil cultivator pass.

Sulphur fertilizer used in the field experiments was Sulfammo 25, which contains 25% N, 31% SO_3 and 2% MgO. The rates of Sulfammo 25 in the experimental variants with sulphur were the following: 77.5 kg/ha for the variant with 24 kg SO_3/ha, 154.8 kg/ha for the variant with 48 kg SO_3/ha, and 232.2 kg/ha for the variant with 72 kg SO_3/ha.

The total nitrogen rate assured for the entire experimental field was of 142 kg/ha, the difference of the nitrogen rate coming from the complex fertilizer 15:15:15 + Sulfammo 25 being assured by applying ammonium nitrate (33.5% N) for each experimental variant.

Sulfammo 25 fertilizer was applied in the growth stage BBCH-20 (no side shoots), and the ammonium nitrate was applied in the growth stage BBCH-31 (1 visibly extended internode), except for the control variant for which the ammonium nitrate was applied in two rates, respectively 100 kg/ha commercial product in the growth stage BBCH-20 and 145 kg/ha commercial product in the growth stage BBCH-31.

The previous crop was winter wheat, and the tillage was a minimum tillage system which entailed: one disc harrow pass at 10 cm depth for stubble cultivation followed by soil fertilization with 15:15:15 fertilizer; one cultivator pass at 20 cm depth to mobilize the soil and better distribute depth wise the applied fertilizer. For oilseed rape crops seedbed preparation is essential as the seed must be planted at 2-3 cm depth maximum and it requires optimal conditions for the small seeds to germinate. So, seedbed preparation received special attention, this being prepared with a fine cultivator.

The used planting rate was, as recommended by the seed producer, 50 germinal seeds per square meter. The planting date was for every year the beginning of the last decade of August, this being the beginning of the optimal planting interval for oilseed rape in the area of research.

Depending on row spacing two types of planting equipment was used: for 12.5 and 25 cm row spacing a classical seed drill was used, while for 37.5, 45 and 70 cm row spacing an 8 row planter was used.

Weed control was realized through the use of a graminicide, Select Super 0.8 L/ha, containing 120 g/l of cletodim, especially to eliminate volunteer wheat. For dicotiledonate weeds control it was used the herbicide Korvetto 1 L/ha containing halauxifen-metil (5 g/l) and clopyralid (120 g/l).

Oilseed rape is a very sensitive crop to pest and therefore a thorough program of insecticide application was required: in September to counter an attack by *Athalia rosae* larvae together with Aphids; one insecticide based on acetamiprid against *Meligethes aeneus* and *Epicometis hirta* in late April/early May.

Fungal pathogens are an important limitative factors for oilseed rape yield and thus the first fungicide used was Caramba Turbo containing metconazole (30 g/l) and mepiquat chloride (210 g/l), in a rate of 1 l/ha and applied in early spring in order to counter phoma (*Plenodomus lingam*) and early sclerotinia rot infections (*Sclerotinia sclerotiorum*). A second fungicide application of Pictor containing dimoxystrobin (200 g/l) and boscalid (200 g/l) used in 0.5 l/ha rate at flowering time ensures protection against sclerotinia rot infections (*Sclerotinia sclerotiorum*) and extended plant life through stay green effect from the contained strobilurins.

The experimental plots were harvested at 13% moisture content of the seeds in order to minimize silique dehiscence effects on yield.

The research was focused mainly on yield and elements that impact yield development. Therefore, the determinations made in the experimental plots involved the following elements:

- Yield (kg/ha);
- Thousand grain weight (g);
- Number of branches per plant;
- Number of pods per branch;
- Number of pods per plant;
- Number of seeds per pod;
- Number of seeds per plant.

To determine the statistical significance of the set of primary data, obtained in the field experiments, it was used the test package from the excel statistical analysis extension XLSTAT, firstly to determine correlation between the studied variables based on the values of the R^2 coefficient. Further analysis was conducted using Fisher test to strengthen the analysis of differences between variants and control (P-value < 0.05) regardless of statistical sample size. Dunett test was used to compare variant by variant with the control in order to identify which variants offer the best solution in terms of yield increases. Tukey test was used to test for significance of all pairwise comparisons, which compares each variant against each other and against control variant in order to find the most statistically significant variants which are the best options to propose as recommendations to be used in practice.

RESULTS AND DISCUSSIONS

Variant with 37.5 cm row spacing and 72 kg SO_3 per hectare generated the highest statistically significant yield of 3,689 kg per hectare with an increase of 857 kg/ha compared to the control variant yield of 2,832 kg per hectare. All tested variants have had significant increases in yield comparing to the control variant, except the variant with 24 kg of SO_3 and 70 cm spacing (Table 3).

The highest yields obtained at Sulphur rate of 72 kg SO_3 per hectare are according to the findings reported by different authors, even there are some differences in the reported results. Thus, Malhi et al. (2005) reported that applying of 15-30 kg S ha^{-1} was sufficient to prevent S deficiency in canola on most of the S-deficient soils. Grant et al. (2012) reported that 15-60 kg S ha^{-1} as sulfate usually optimize the seed yields of both open-pollinated and hybrid oilseed rape. Wielebski (2008) reported a significantly higher yield after application dose of sulphur 10-30 kg.ha^{-1}. Vaseghi et al. (2013) reported that several experiments proved an increased seed yield at 40 kg.ha^{-1} of sulphur.

Thousand seed weight is an important element in assessing the quality and quantity of yields. Statistical analysis highlights that two variants with 72 kg of SO_3 per ha with 45 or 37.5 cm row spacing have led to significant increases in thousand seed weight compared to control and the other variants (Table 4).

Table 3. Yield (kg/ha) results and their significance

Category	LS means (Average Yield 21-23)	Significance Groups						
72 kg SO_3-37.5 cm	3689.0	A						
72 kg SO_3-45 cm	3602.7		B					
48 kg SO_3-37.5 cm	3403.0			C				
48 kg SO_3-45 cm	3389.0			C				
72 kg SO_3-25 cm	3349.5			C				
72 kg SO_3-70 cm	3280.5				D			
24 kg SO_3-37.5 cm	3259.0				D			
24 kg SO_3-45 cm	3167.7					E		
48 kg SO_3-25 cm	3061.0						F	
24 kg SO_3-25 cm	3010.0						F	
48 kg SO_3-70 cm	2991.7						F	
24 kg SO_3-70 cm	2856.0							G
Control	2832.0							G

Table 4. Significant increases in thousand seed weight

Contrast	Difference	Standardized difference	Critical value	Critical difference	Pr > Diff	Significant
Control vs 72 kg SO_3 - 45 cm	-4.337	-3.541	2.905	3.558	0.010	Yes
Control vs 72 kg SO_3 - 37.5 cm	-4.240	-3.462	2.905	3.558	0.012	Yes

The total number of branches per plant positively correlates with the quantity of applied sulphur. At the same time row spacing of 45 cm between rows is favoured as it leads to the highest number of branches per plant with a significant impact for application of 72 kg SO_3/ha as well as for application of 48 kg SO_3/ha (Table 5). On average 45 cm row spacing variants and medium to high sulphur fertilization lead to an extra 3 branches of oilseed rape to develop. The obtained results are according to those obtained by Ahmad et al. (2011) by applying sulphur fertilizer in a rate of 40 kg/ha a.s. in oilseed rape crops which has led to an improvement of plant architecture as it was observed an increase in the overall number of lateral branches per plant, which positively impacted the number of siliques per plant.

Table 5. Number of branches per plant

Category	No of branches per plant
72 kg SO_3 - 45 cm	16.86
48 kg SO_3 - 45 cm	16.12
48 kg SO_3 - 70 cm	16.09
72 kg SO_3 - 37.5 cm	14.90
72 kg SO_3 - 70 cm	14.41
24 kg SO_3 - 37.5 cm	14.24
24 kg SO_3 - 70 cm	14.18
24 kg SO_3 - 45 cm	14.18
48 kg SO_3 - 37.5 cm	14.02
24 kg SO_3 - 25 cm	13.85
72 kg SO_3 - 25 cm	13.77
48 kg SO_3 - 25 cm	13.680
Control	13.430

Sulphur content positively correlates with the number of seeds per silique as it facilitates better viability of the flowers and lower stress effect in the late flowering period (Table 6). Medium row spacing of 45 cm or 37.5 cm allows for better plant canopy development and architecture facilitating surpassing stress at pollination time.

45 and 37.5 cm row spacing with high sulphur fertilization (72 kg SO_3/ha) have led to the highest significant increase compared to control regarding the number of seeds per silique for both row spacing.

The number of seeds per silique is a sensitive subject as draught, hot air temperature and more critically lack of water will greatly impact the number of pollinated ovules.

Table 6. Number of seeds per silique variation

Category	No of seed per silique
72 kg SO_3 - 45 cm	11.48
72 kg SO_3 - 37.5 cm	11.45
48 kg SO_3 - 45 cm	11.40
72 kg SO_3 - 25 cm	11.30
48 kg SO_3 - 37.5 cm	10.98
48 kg SO_3 - 25 cm	10.87
24 kg SO_3 - 45 cm	10.67
24 kg SO_3 - 37.5 cm	10.26
72 kg SO_3 - 70 cm	10.05
48 kg SO_3 - 70 cm	9.87
24 kg SO_3 - 70 cm	9.73
24 kg SO_3 - 25 cm	9.67
Control	9.03

The number of siliques per branch is also impacted by sulphur fertilization with its role in the stress management mechanism of the plant. One can observe slight increases in the number of siliques per branch in the case of medium and high sulphur fertilization variant (Table 7). The number of siliques per branch is increased when using 45 or 37.5 cm row spacing by 16.93% and 18.6%, respectively. So, in terms of row spacing, medium spacing variants offered the best compromise.

Draught, hot air temperature and more critically lack of water will greatly impact the formation of the number of flowering buds per branch and thus the number of siliques per branch.

Table 7. Number of siliques per branch variation

Category	No of siliques per branch
72 kg SO_3 - 37.5 cm	13.45
48 kg SO_3 - 45 cm	13.32
72 kg SO_3 - 45 cm	13.25
48 kg SO_3 - 37.5 cm	13.05
48 kg SO_3 - 25 cm	12.99
24 kg SO_3 - 45 cm	12.94
24 kg SO_3 - 37.5 cm	12.75
24 kg SO_3 - 25 cm	12.55
72 kg SO_3 - 25 cm	12.51
72 kg SO_3 - 70 cm	12.14
48 kg SO_3 - 70 cm	12.02
24 kg SO_3 - 70 cm	11.97
Control	11.34

The total number of siliques per plant is positively correlated with a high degree of correlation with applied sulphur rate (Table 8).

The highest number of siliques per plant was obtained in variants with 72 kg SO_3/ha (Table 9). The optimal row spacing for increasing the number of silique per plant is 37.5 cm or 45 cm. The total number of siliques per plant is impacted by the statistically significant variants with 37.5 cm and 45 cm between rows and 72 kg SO_3/ha. Practically, all variants have outperformed quantitatively and statistically the control variant.

Table 8. Regression of between factors regarding number of siliques per plant

Observations	52
Sum of weights	52
DF	39
R^2	0.993
Adjusted R^2	0.991

The number of seeds per plant is significantly higher compared to the control variant for all teste variants.

The highest number of seeds per plant was obtained in variants with high sulphur fertilization of 72 kg SO_3/ha. Also, the number of seeds per plant was higher for variants with medium to low row spacing: 37.5 cm, 45 cm, and 25 cm (Table 10).

Variants with 37.5 cm and 45 cm between rows with high sulphur fertilization (72 kg SO_3/ha) lead to the highest statistically significant increase in the number of seeds per plant by 68.36% and 67.10% respectively compared to the control variant.

Table 9. Number of siliques per plant variation

Category	Means (No of silique per plant)	Significance Groups
72 kg SO_3 - 37.5 cm	266.35	A
72 kg SO_3 - 45 cm	260.79	A
48 kg SO_3 - 37.5 cm	252.55	B
48 kg SO_3 - 45 cm	246.70	C
72 kg SO_3 - 25 cm	237.03	D
72 kg SO_3 - 70 cm	226.67	E
24 kg SO_3 - 37.5 cm	223.45	EF
24 kg SO_3 - 45 cm	219.30	FG
48 kg SO_3 - 25 cm	215.50	G
24 kg SO_3 - 25 cm	206.75	H
48 kg SO_3 - 70 cm	202.10	HI
24 kg SO_3 - 70 cm	198.40	I
Control	184.50	J

Table 10. Number of seeds per plant significance per variant

Contrast	Difference	Standardized difference	Critical value	Critical difference	Pr > Diff	Significant
Control 1 vs 72 kg SO_3 - 37.5 cm	-1342.00	-821.804	2.905	4.744	<0.0001	Yes
Control 1 vs 72 kg SO_3 - 45 cm	-1317.12	-806.572	2.905	4.744	<0.0001	Yes
Control 1 vs 72 kg SO_3 - 25 cm	-1021.76	-625.698	2.905	4.744	<0.0001	Yes
Control 1 vs 48 kg SO_3 - 37.5 cm	-885.70	-542.378	2.905	4.744	<0.0001	Yes
Control 1 vs 48 kg SO_3 - 45 cm	-847.20	-518.802	2.905	4.744	<0.0001	Yes
Control 1 vs 72 kg SO_3 - 70 cm	-794.79	-486.710	2.905	4.744	<0.0001	Yes
Control 1 vs 24 kg SO_3 - 37.5 cm	-451.20	-276.302	2.905	4.744	<0.0001	Yes
Control 1 vs 24 kg SO_3 - 45 cm	-406.00	-248.623	2.905	4.744	<0.0001	Yes
Control 1 vs 48 kg SO_3 - 25 cm	-280.00	-171.464	2.905	4.744	<0.0001	Yes
Control 1 vs 48 kg SO_3 - 70 cm	-180.00	-110.227	2.905	4.744	<0.0001	Yes
Control 1 vs 24 kg SO_3 - 25 cm	-102.90	-63.013	2.905	4.744	<0.0001	Yes
Control 1 vs 24 kg SO_3 - 70 cm	-22.00	-13.472	2.905	4.744	<0.0001	Yes

CONCLUSIONS

Following the obtained results in the specific conditions of NE Romania and for the studied hybrid, it can be concluded that the use of 37.5 cm row spacing is the optimal solution to find the best equilibrium between plant architecture development and development of yield elements. If there is no technical mean to modify planters the next best solution would be the use of 45 cm row spacing which is commonly used for soybean planting and other technical plants in order to obtain adequate yielding elements development.

In terms of sulphur fertilization it is paramount to not neglect it as low sulphur availability will lead to significant losses of yield. Optimal rate of sulphur for the studied conditions was of 72 kg SO_3/ha.

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EFFECT OF FUNGICIDES ON DEOXYNIVALENOL CONTENT IN WINTER WHEAT (*Triticum aestivum* L.)

Adelina HARIZANOVA, Vanya IVANOVA

Agricultural University of Plovdiv, 12 Mendeleev Blvd, Plovdiv, Bulgaria

Corresponding author email: aharizanova@yahoo.com

Abstract

The aim of the present study was to analyze the effect of four fungicidal products on Gibberella ear rot (Fusarium graminearum) on yield quality and quantity of winter wheat (Triticum aestivum L.), cv. Venka 1 and choose the more appropriate application rate. The field experiment was performed in 2022 in Ruse region, Bulgaria. A local variety of winter wheat sensitive to Fusarium ear rot was chosen. The test product (Prothiconazole 174 g/l + Pydiflumetofen 60 g/l) was applied in three dose rates – 0.9, 1.2, and 1,5 l/ha. As reference products, three registered fungicides were selected: Prosaro 420 SC (Prothioconazole 210 g/l + Tebuconazole 210 g/l) - 1 l/ha, Delaro (Prothioconazole 125 g/l + Trifloxystrobin 125 g/l) - 1 l/ha, and Input (Prothioconazole 160 g/l + Spiroxamine 300 g/l) - 1 l/ha and 1.25 l/ha. Nine variants, including untreated inoculated control and seven different treatments, were analyzed. The results showed a significant decrease in the disease severity and incidence after applying all the products and a reduction of deoxynivalenol content in the grain. The yield of all the fungicide treated plots increased.

Key words: *fungicide, Fusarium graminearum, mycotoxins, winter wheat, yield.*

INTRODUCTION

Wheat is one of the most commonly grown crops worldwide (Khaneghah et al., 2018). The wheat-based products could be categorized as the primary sources of protein and energy of vegetable origin (Jones, 2005). Wheat grains are usually processed into flours which are used for culinary purposes to produce bread, pasta, and cookies or could be used in other industrial sectors. Wheat is highly relevant from both economic and nutritional points of view, but unfortunately, it is susceptible to fungal contamination. The fungal disease may result in the reduction of the grain quality of wheat and the production of mycotoxins (toxic secondary metabolites that are formed by fungi) (Filtenborg et al., 1996).

One of the diseases of great economic importance in wheat is Fusarium head blight (FHB) (Edwards, 2004). The infection causes a reduced yield because of shrunken grains and results in poor milling and malting quality and the contamination of grains with mycotoxins. The most important pathogens of wheat causing FHB are *Fusarium graminearum* and *F. culmorum*. They are more pathogenic than other species and they produce higher amounts of mycotoxins, especially deoxynivalenol (DON). During late spring, the conidia are splash dispersed via rainfall. The dry weather usually promotes sporulation (Edwards, 2004). Mycotoxins are a large group of substances with a small molecular weight with diverse structures. The main producer of these compounds is the secondary metabolism of some filamentous fungi or molds. They are able, under suitable temperature and humidity conditions, to develop on various foods and feeds and to cause serious risks to human and animal health (Zain, 2010). Deoxynivalenol (DON) is a mycotoxin that contaminates grains, cereal-based food, and feed. It belongs to the group of trichothecenes and engenders intense gastrointestinal adverse effects like vomiting (emesis) in animals and humans. The long-term dietary exposure to DON in animals leads to weight gain suppression, altered nutritional efficiency, and anorexia (EFSA 2013). Sensitivity to DON depends on the specific metabolism, the absorption, circulation, and elimination of DON by the organism (Sobrova et al., 2010). It is observed that some ruminants such as cattle, sheep, goats, and deer are less sensitive to the negative effects of mycotoxins than the non-ruminant organisms. However, if the animals consume mycotoxin-contaminated feed for a long period, this could alter the growth, reproduction, and quality of production (milk, beef, or wool) (Hussein and Brasel, 2001). Therefore, according to Morgavi & Riley (2007), DON is the primary compound, which is responsible for economic losses due to the reduction of performance in animal husbandry. The consumption of DON-contaminated wheat and wheat-based products, such as bread and pasta, could also endanger human health (Khaneghah et al., 2018). The content of mycotoxins in plants can be influenced using different agro technical practices, the most successful of which is the use of fungicides. Fusarium head blight infection occurs at flowering and numerous studies have shown that fungicide application is most effective when applied from early to mid-flowering (Edwards, 2022).

MATERIALS AND METHODS

Experimental design

The field experiment was set up in Ruse Region, Bulgaria in 2022. *Triticum aestivum* L. cv. Venka 1 was selected as a test object. The experiment was set up using the block plot method. Each plot was with the size of 24 m^2. The plants were sown on 27 October 2021 with a row spacing of 3 cm and spacing within row of 10 cm. The sowing rate was 250 kg/ha of seeds. The date of emergence was 18 November 2021. The yield was harvested on 12 July 2022.

Artificial inoculation

The test plots were artificially contaminated with *Fusarium graminearum*. Inoculation of the disease was conducted using a spraying spore suspension method. Conidia solution was prepared at concentration of 10^5 to 10^6 conidia/ml to use at a final spray concentration of 10^3 to 10^4 conidia/ml. The inoculum was applied one day before the application of the test products, at BBCH stage 63 (flowering), as flowering is the most sensitive growing stage for artificial inoculation.

Application data

The test plots include inoculated untreated control and eight different treatments. The control plants were not treated with a fungicidal product. The test product contains Prothioconazole 174 g/l + Pydiflumetofen 60 g/l, and it was applied in three dose rates to select the most appropriate application dose. As reference products, three already registered fungicides were used. These were Prosaro 420 SC (applied in one dose), Delaro (applied in two doses), and Input (Bayer Crop Sciences), applied also in two dose rates. Prosaro 420 SC contains Prothioconazole 210 g/l + Tebuconazole 210 g/l, Delaro contains Prothioconazole 125 g/l + Trifloxystrobin 125 g/l. Input contains 160 g/l Prothioconazole + 300 g/l Spiroxamine. The list of the experimental variants included nine variants and is given in Table 1. All the products were applied one day after the artificial inoculation in BBCH 63 (flowering). The spray volume was 500l/ha.

Table 1. List of treatments

Variant	Description
1	Untreated control
2	Test Product 0.9 l/ha
3	Test Product 1.2 l/ha
4	Test Product 1.5 l/ha
5	Prosaro 420 SC 1 l/ha
6	Delaro 1 l/ha
7	Delaro 1.25 l/ha
8	Input 1 l/ha
9	Input 1.25 l/ha

Mycotoxin content estimation

The mycotoxin concentration was estimated using high-performance liquid chromatography (HPLC) with tandem mass spectrometry (MS/MS) meeting VLM 92 2010.

Statistical analyses

The data are presented as mean of 4 replicates. The experimental results were statistically processed with the SPSS program using a one-way ANOVA dispersion analysis and Duncan's comparative method, with the validity of the differences determined at a 95% significance level. The different letters (a, b, c, d) after the mean value show statistically significant differences between the variants.

RESULTS AND DISCUSSIONS

Data about the *Fusarium graminearum* severity is presented in Figure 1. It is seen that the highest disease severity is observed in variant 1 - the untreated control (about 13.7%). On all of the test plots, which were treated with pesticides, there was a great reduction in the disease severity compared to the control. In variant 2 and variant 6, disease severity was about 2.1 and respectively 2.3%, and in variant 3 - less than 2%. In variants 4, 5, 7, 8, and 9, the percentage of the infected area was lower that 1%. The results show that the application of the test product is able to reduce significantly the disease severity in comparison to the untreated plots. The best result is obtained using the higher rate of application of 1.5 l/ha. The lowest disease severity is observed on variant 9, where Input in the dose of 1.25 l/ha was applied.

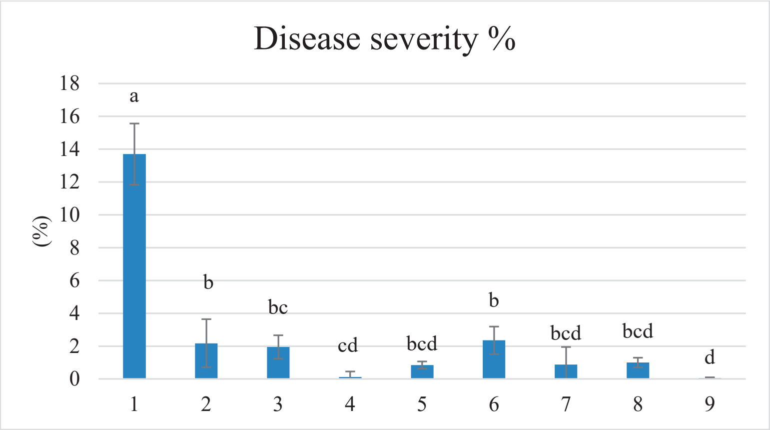

Figure 1. Disease severity (% area) of *Fusarium graminearum* in winter wheat after artificial contamination and pesticide application

The disease incidence measurement shows that the highest number of infected plants was observed on the control plots (Figure 2). Almost 45% of the control plants had disease symptoms. The number of the infected plants treated with the test product was significantly lower. Only 12% in variant 2 and about 10.5% in variant 3, respectively. The lowest rate of disease incidence was measured on the plots treated with 1.5 l of the test product (variant 4) and the plants treated with Input 1.25 l/ha (variant 9). On the other experimental plots, the disease severity varied between 9 and 4%.

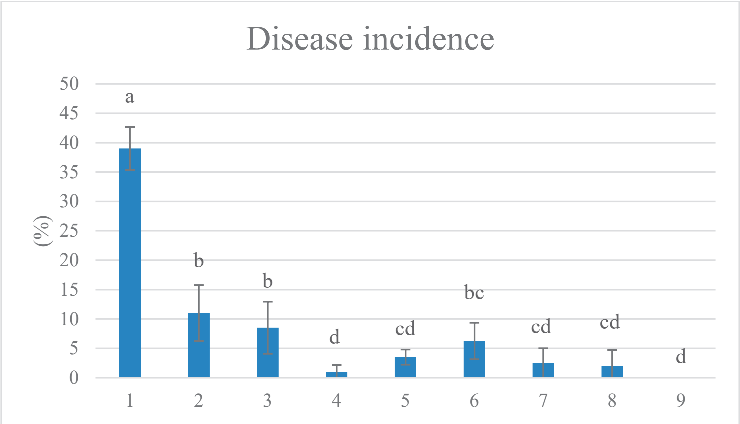

Figure 2. Disease incidence (%) of *Fusarium graminearum* in winter wheat after artificial contamination and pesticide application

It was expected that if the disease severity and incidence were so significantly reduced after the application of the fungicidal products, the content of mycotoxins would be reduced too. The analyses of the mycotoxin content are presented in Figure 3. All the variants, which were treated with a fungicide, showed a significantly lower mycotoxin content. The content of deoxynivalenol is presented in Figure 3.

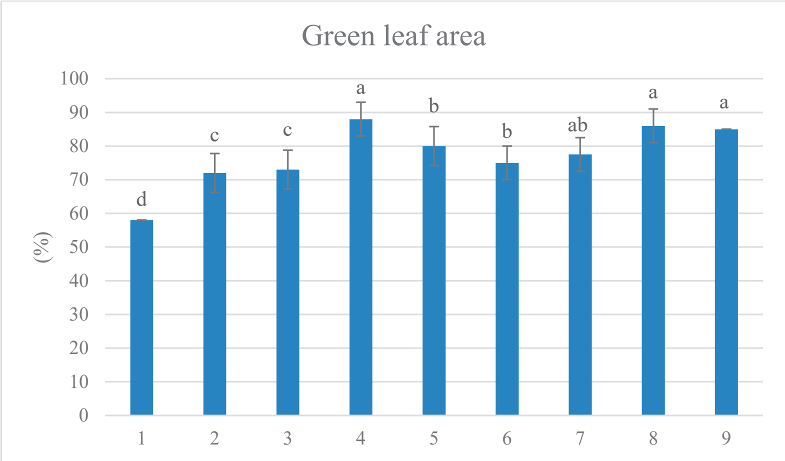

Figure 3. Green leaf area % in winter wheat after the contamination with *Fusarium graminearum* and pesticide application

According to Pepler et al. (2005), the use of fungicides is associated with yield enhancement, and the reason for that is the improvement of photosynthesis during grain filling. The physiological status of the green leaf area is maintained by restricting the development of known pathogens (Ruske et al., 2003) or even unknown pathogens which are hardly identified in the field (Bertelsen et al., 2001). Sometimes the positive effect of the pesticide application is due to the direct physiological effects of the fungicide itself on the plant (Grossmann and Retzlaff, 1997). The percent of the green leaf area, which had no visible symptoms of the disease, is presented in Figure 3. The lowest percent of green area is measured in the untreated control and in variant 9. The highest percent is measured in variant 4 and variant 8. According to Morgavi & Riley (2007), mycotoxins, especially DON, are characterized as a major cause of economic loss due to reduced performance, although they are not considered acutely toxic to farm animals. In concern to human safety, The Scientific Committee on Food (SCF) established a temporary Tolerable Daily Intake (TDI) of 1 µg/kg body weight (b.w.) per day. This was established after a 2-year feeding study in mice with decreased gain body weight, based on a No Observed Adverse Effect Level (NOAEL) of 100 µg/kg b.w. per day (SCF, 2002). In 2010, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) proposed an Acute Reference Dose (ARfD) at 8 µg/kg body weight. According to EFSA (2013), high consumers and young children were exposed to DON at levels close to or even higher than the TDI (EFSA, 2013).

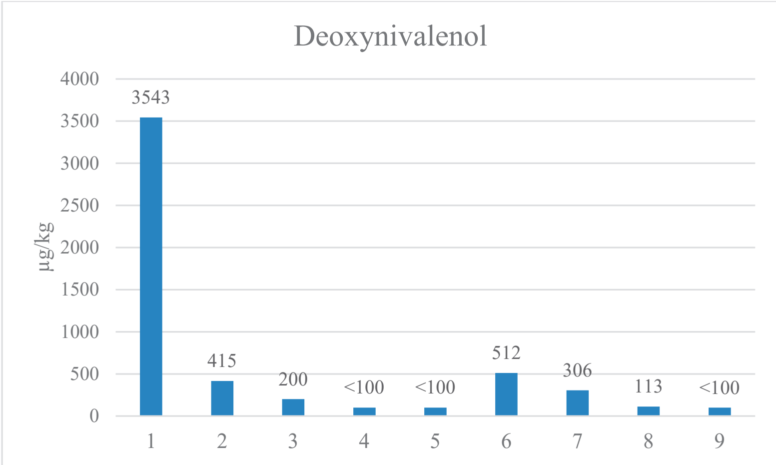

Figure 4. Content of deoxynivalenol (µg/kg) of winter wheat after the contamination with *Fusarium graminearum* and pesticide application

In this regard, the minimum DON content measured in the current experiment was 100 µg/kg. The content of DON is presented in Figure 4. It is seen that the lowest amounts of mycotoxins are observed in variants 4, 8, and 9, where the content of deoxynivalenol is so small that it could not be measured (as the methodology of the accredited laboratory detects concentrations of a minimum of 100 µg/kg). The pesticide application reduced the amounts of the toxic secondary metabolite to very low levels. The rate of reduction depends on the product and its application rate.

It is observed that the content of mycotoxins is reduced between 7 and 50 times compared to the control. As expected, the lowest disease severity and incidence resulted in a better yield. The results of the yield of winter wheat are presented in Figure 5. Compared to the untreated control, the pesticide application yielded an increase in all the test variants where fungicides were applied. The highest yields are measured in variant 4 and variant 8, where the test product in a dose of 1.5 l/ha and Input in a dose of 1 l/ha, respectively, were used.

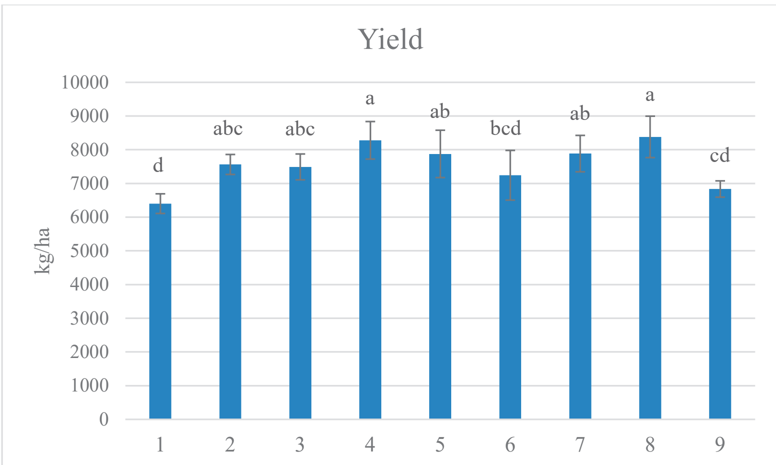

Figure 5. Yield of winter wheat after the contamination with *Fusarium graminearum* and pesticide application

There are few scientific works on the efficacy of pydiflumetofen (succinate dehydrogenase inhibitor) against FHB. They observe either its effect as a solo product or as a co-formulation with propiconazole (Singh et al., 2020; Xia et al., 2021). According to Edwards & Godley (2010) the pesticide Proline containing propiconazole was able to reduce the infection of head blight on oats and the content of DON, respectively. In the research of Edwards (2022) it is pointed out that the new fungicide, containing pydiflumetofen in a co-formulation with prothioconazole (triazole), was significantly better at reducing FHB disease and resulted in a reduction of DON contamination of grain than either pydiflumetofen or prothioconazole alone.

CONCLUSIONS

The current experiment aimed at exploring the effect of different pesticides on yield and the content of mycotoxins, namely DON, in winter after the artificial inoculation with *Fusarium graminearum*. The results show that all the tested fungicides could reduce the disease severity and incidence of *Fusarium graminearum* on winter wheat. As a result, the content of mycotoxins in grain was significantly lower in all the test variants compared to the untreated control. This led to an increase in the yield in the plots where fungicides were applied. The highest yield was observed in variant 4 after applying the test product containing Prothioconazole 174 g/l + Pydiflumetofen 60 g/l in a dose of 1.5 l/ha and in variant 8, where Input was applied in a dose of 1 l/ha. The strongest reduction of the mycotoxin content was measured in variant 4, variant 8, and variant 9, where the test product in a dose of 1.5 l/ha, Input in a dose of 1 l/ha, and a dose of 1.25 l/ha was applied.

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EFFICIENCY OF WINTER WHEAT CULTIVATION AFTER SPRING BARLEY IN THE NORTHERN STEPPE OF UKRAINE

Iryna HASANOVA[1], Marharyta YERASHOVA[1], Nataliia NOZDRINA[2], Oleksandr PEDASH[1], Alla KULYK[1]

[1]State Establishment Institute of Grain Crops of National Academy of Agrarian Sciences of Ukraine, 14 Volodymyr Vernadsky Street, Dnipro, 49009, Ukraine
[2]Dnipro State Agrarian and Economic University, 25 Serhii Yefremov Street, Dnipro, 49000, Ukraine

Corresponding author email: natalija_87@ukr.net

Abstract

The influence of environmental conditions and mineral fertilizers on the efficiency of growing winter wheat after spring barley was revealed. It was found that nitrogen feedings after stubble previous crop in the spring-summer period of growing season does not lead to an increase in plant productivity in dry years. In favorable years in terms of moisture supply yields and economic indicators of cultivation increased. Thus, on average for 2016-2018, the highest yield and net income from the growing winter wheat after spring barley were obtained in the experimental variants, where feeding with ammonium nitrate N_{60} on the background of $N_{60}P_{60}K_{30}$ was carried out on freeze-thawed soil or in two terms: N_{60} on freeze-thawed soil and N_{30} locally at the end of the tillering stage of plants. According to the fertilization variant, the yield and net income varied within 4.78-5.47 t/ha and 16,041-19,949 UAH/ha, as well as 5.03-5.62 t/ha and 16,752-21,347 UAH/ha.

***Key words**: winter wheat, variety, previous crop - spring barley, nitrogen feedings, yield, economic efficiency.*

INTRODUCTION

Under contemporary social and economic conditions, one of the significant tasks for the Ukrainian agricultural sector is the increase and stabilization in grain yield, mainly for the principal food crop - winter wheat *(Triticum aestivum* L.). Its harvest is influenced by the number of factors. Among the uncontrollable factors one can name as follows: solar radiation, air temperature, rainfall, the autumn vegetation period duration, the time of spring vegetation resumption, etc. (Zhemela & Musatov, 1989).
The last years in the Ukrainian Northern Steppe zone saw mostly unfavourable conditions for winter wheat in pre-sowing and after-sowing periods of vegetations. High air temperatures and insufficient rainfall at these periods resulted in air and soil droughts, which prevented winter wheat seedlings from appearing timely and delayed the development of these plants. The conditions were more severe for those winter wheat which had been sown after such non-fallow previous crops as stubble crops and oil cultures since they reduce the opportunities for moisture accumulation and preservation within the soil before sowing. This moisture is necessary for obtaining even stands of seedlings and further sufficient development of plants (Cherenkov et al., 2015).
According to the researchers, good seedlings of winter wheat require that the productive moisture reserves in the soil layer of 0-20 cm deep is to be at least 20 mm (Lichikaki, 1958), while that of 0-10 cm is to possess at least 10 mm (Kulyk, 2008). At the lower values of soil productive moisture, the decreases both in the sustainability of seedlings and in their even stands at the beginning of the plant development are observed. It is determined that depending on hydro and thermal conditions, the period between sowing and seedlings to appear varies from 6 days to 90 days in different years, according to the researches (Klimov & Povzik, 1980). Moreover, it is defined that the moisture shortage is not cured by wider range of the mineral fertilizers, plant growth stimulators and pesticides (Zubets et al., 2010).
The better developed plants in autumn, the more resistant they are to droughts and they grow higher yield. Late seedlings, poorly developed seedlings, seedlings without tillering since

autumn are usually of low productiveness. Under conditions of the Southern Steppe, the years of dry autumns have registered the 8.2-18.0% decrease in winter wheat yield as compared against the years with favorable autumns. Further, at early spring, the wheat plants have longer tillering, develop crown roots and ensure sufficient grain yield even if sowing has been late. The late spring produces an adverse effect on the poorly developed seedlings: their even stands gradually become less frequent and form low grain yield (Netis & Onufran, 2016).

Along with the mentioned, the researchers consider that the scientifically grounded approaches towards the choice of the wheat variety, the soil cultivation, the previous crop, the sowing time, the fertilizing system and the protection against pests and diseases are capable of increasing the wheat productivity significantly and respectively wheat growing even under disadvantageous conditions (Cherenkov et al., 2015; Mykhalska & Shvartau, 2018; Solodushko, 2021).

Furthermore, one of the most important techniques of obtaining excellent yields of winter wheat grains along with their high-quality is proved to be the right choice of the previous crop for the certain crop. The long-term research carried out at Institute of Grain Crops of National Academy of Agrarian Sciences of Ukraine has pointed out black fallow, full fallow, leguminous crops, perennial grasses as the best previous crops (Hodulian & Bardunova, 1967; Pikush et al., 1992). However, the last years sharply decreased the share of these previous crops in the Ukrainian Steppe zone and increased the sowing areas for winter wheat after stubble crops, sunflowers, sorghum for grain, which drastically deplete soil taking away its moisture, nutrients and other components (Zubets et al., 2010; Cherenkov et al., 2018). This means that the conditions for winter wheat seed germination, their development in the autumn period and vegetation period deteriorate, the frost-resistance and the winter hardiness in wheat reduce, which results in less dense seedling stands and lower grain yield.

The yield and the quality of winter wheat grains considerably depend on the availability of the mineral elements for the plants during the whole period of their vegetation. When applying the fertilizers for winter wheat plants one also should take into account the previous crop, soil and climate conditions, and the specific character of the interrelation effect of the weather conditions in a certain case (Maadi et al., 2012; Netis, 2011). Fertilizers are one of the most expensive components for winter wheat growing that is why the determination of their rational norms to be applied is the urgent task of the contemporary science.

The aim of the current research is to define the impact of the weather and the mineral nutrition on the yield and the economic efficiency for winter wheat growing after spring barley under conditions of the Ukrainian Northern Steppe.

MATERIALS AND METHODS

The reported research is the long-term studies on winter wheat sown after spring barley as a previous crop on common black soil, low in humus, full cross-section. The research has been carried out at State Establishment Institute of Grain Crops NAAS of Ukraine. The sowing time was optimal for Northern Steppe zone - 20 September, the rate of seeding was 5,000,000 viable seeds/ha, the depth of seed wrapping was 5-6 cm. The norm for fertilizers applied was $N_{60}P_{60}K_{30}$. During the years of the research, on the background of pre-sowing fertilizer, spring-summer nitrogen feedings were introduced by the fertilizers as follows: ammonium nitrate, urea ammonium nitrate mixture (UAN-32), carbamide. Ammonium nitrate was put randomly on the soil surface and locally by means of the seeders; carbamide and UAN-32 were introduced by spraying of vegetative plants with backpack sprayers.

The field experiments and wheat grain quality determination were performed in accordance with the applicable standards and approved methods (DSTU 3768-2019; Volkodav, 2001). Wheat plant development and growth were monitored directly by phenological observations. The yield was determined via continuous threshing with “Sampo-500” combine on each plot and weighing with the subsequent calculation per 1 ha (Dospekhov, 1985).

The economic efficiency on the variations in nitrogen supplements for winter wheat growing was defined by utilizing the average price information on resources and grain in the years of the research. The principal criteria of economic efficiency were determined with yield indicators and we also took into account the grain quality after each agricultural technique application. The calculation and monitoring were performed based on the typical flow charts of wheat growing and conventional techniques (Cherenkov et al., 2017; Hyrka et al., 2019).

RESULTS AND DISCUSSIONS

In Steppe zone, the best productivity and frost resistance have been shown by those winter wheat plants, which managed to acquire 3-5 shoots before completing autumn vegetation. For obtaining such a number of shoots, the autumn period of the plant growing and development should be within the range of 55-65 days, while the sum of the effective temperatures (> 5°C) - 250-300°C. The duration of the indicated period is rather long for the wheat plants to accumulate the sufficient amount of plastic substances and as the result the plants become more capable of resisting the unfavorable conditions of winter period as well as those in spring and summer periods (Cherenkov et al., 2021).

Moreover, apart from the mentioned, the necessary constituent of winter wheat growing is availability of the productive rainfall during the period of plant vegetation in autumn. During the many years, the average amounts of the rainfall in the zone of our research for the period from sowing to vegetation completion in autumn (sowing date of 20 September and the average autumn vegetation is 60 days) have been approximately 80 mm.

The analysis on the last 13 years of the research has shown the significant differences in weather conditions between them in terms of the duration of autumn vegetation, the sum of the accumulated effective temperatures for the period and moisturizing conditions. The term of the autumn vegetation final completion was varying from 6th November (early completion of vegetation in 2011 and 2018) to 1st December and 4th December (respectively, late term of vegetation completion in 2010 and 2012). Further, the sum of the effective temperatures not always was dependent on the duration of autumn vegetation period. Thus, in 2018, the sum of the effective temperatures was 316.4°C with autumn vegetation period of 47 days, while, for instance, in 2010, the dedicated period was 25 days longer, but the sum of the effective temperatures was 295.9°C. The highest values of this parameter were in 2020 - 420.8°C, in 2012 - 389.6°C and in 2019 - 325.1°C. The lowest value was in 2016 and totaled - 181.7°C (Table 1).

Table 1. Hydro and thermal conditions of winter wheat autumn vegetation in 2010-2022

Year	The date of the final termination of autumn vegetation	Term of the duration of autumn vegetation, days	The sum of the effective temperatures during the period «sowing-termination of autumn vegetation», °C	The rainfall amount during the period «sowing-termination of autumn vegetation», mm
2010	1st December	72	295.9	132.1
2011	6th November	47	240.1	16.2
2012	4th December	75	389.6	104.4
2013	26th November	67	204.0	118.0
2014	16th November	57	215.1	88.5
2015	24th November	65	294.1	41.7
2016	11th November	52	181.7	140.7
2017	15th November	56	265.0	78.9
2018	6th November	47	316.4	44.9
2019	16th November	57	325.1	96.7
2020	11th November	52	420.8	70.1
2021	10th November	51	200.2	24.3
2022	26th November	67	297.2	138.9

As it has mentioned before, even at the sufficiently encouraging temperature regime, the moisture shortage within the soil during autumn vegetation is capable of producing the adverse effect on the conditions of winter wheat seed germination, its seedling growth and development.

During the years of the observation, the least rainfall during autumn vegetation were in 2011 and totaled only 16.2 mm. Not high was this value in the following years: in 2021 - 24.3 mm, in 2015 - 41.7 mm and in 2018 - 44.9 mm.

In case of poorly developed plants, the crucial in significance for their further development and growth are the conditions of winter period and spring-autumn period. Under advantageous weather conditions, the state of the plants could notably improve and allow good grain yield. The most severe was the winter time for 2011/2012 growing season. The winter wheat sown after spring barley was in the stage of the third leaf and the beginning of tillering at completing its autumn vegetation. The crops were not dense in their stand and were uneven but their state was estimated as sufficient.

In December and at the beginning of January, it was predominantly warm, frequent fogs and rainfall, that hampered plant hardening, caused the losses in the nutrient substances and the decrease in their winter hardiness. The 15 last days of February were very cold and dry. The average daily air temperature on the coldest days was within -11 ... -24°C, that is 6-19°C lower than the normal meteorological one. At the coldest nights, the air temperature went down to -25 … -30°C.

The steady recovery of winter wheat vegetation in 2012 was registered on 3 April, somewhat later than the conventional terms. The supplies of the productive moisture were enough for the non-fallow previous crops. However, in the period from 24 April to the end of June, the season progressed with abnormally hot weather and the lack of rainfall. This speeded up the winter wheat plant development stages. The plants exhibited early yellow leaves on the bottom, poor formation of reproductive organs and worsening crop state due to the drought. The high temperatures caused early ripening of winter wheat and subsequent reduction in the yield was registered, especially after non-fallow previous crops, and in our study it was detected after spring barley. Thus, the complex of the disadvantageous hydro and thermal factors during 2011/2012 growing season impacted the considerable loss in winter wheat grain after all the previous crops.

The following growing seasons (2012/2013 and 2013/2014) were generally good for winter wheat and this influenced the yields. Thus, the average yield per the wheat variety after spring barley as the previous crop on the background of soil conditioning with complete $N_{60}P_{60}K_{30}$ fertilizing but without nitrogen feedings was 2.37 t/ha in 2012, while in 2013 - 4.17 t/ha, in 2014 - 4.73 t/ha (Table 2).

Nitrogen feedings for the plants at their spring-summer vegetation were attributed to the losses in certain cases and rather low economic efficiency with the wheat varieties as compared to the control groups. However, during the next years to come, winter wheat growing with nitrogen feedings allowed higher grain yields and the net incomes.

Table 2. The economic efficiency of winter wheat growing depended on the previous crop and nitrogen feedings under various weather conditions of the seasons under study

Previous crop	2011/2012		2012/2013		2013/2014	
	No feeding - control	Nitrogen feedings	No feeding - control	Nitrogen feedings	No feeding - control	Nitrogen feedings
Spring barley (background $N_{60}P_{60}K_{30}$)	Yield, t/ha					
	2.37	2.24-2.39	4.17	4.38-4.70	4.73	4.98-5.28
	Net income, UAH/ha					
	1,681	-256-620	4,305	5,003-6,331	6,767	6,771-7,982
	Profitability, %					
	28.8	-3.5-9.5	67.6	63.5-80.0	104.5	84.8-99.6
Black fallow (background $P_{60}K_{30}$)	Yield, t/ha					
	3.87	3.95-4.27	6.69	6.73-7.40	6.77	6.90-7.23
	Net income, UAH/ha					
	5,306	5,361-5,920	7,193	7,758-8,704	7,195	7,762-8,701
	Profitability, %					
	82.6	68.5-83.3	160.4	150.1-172.8	163.5	150.8-169.4

Growing winter wheat after black fallow (on $P_{60}K_{30}$ background) permitted the plants to develop greater vegetative masses and better root systems, even in unfavorable 2012, the yield in the control group was 3.87 t/ha. This result was 1.5 t/ha higher than that obtained under the same condition but after spring barley, though the mineral background was more intensive after the non-fallow previous crop. It worth mentioning that the increases in the yield and economic efficiency were registered even in this disadvantageous year in the trials with nitrogen feedings into black fallow. The yield grew up to 3.95-4.27 t/ha, gross operating profits went up from 5,306 UAH/ha to 5,361-5,920 UAH/ha. The next years were good with their weather conditions (2013 and 2014), the yields after black fallow in the control group were respectively 6.69 t/ha and 6.77 t/ha. The nitrogen feedings during spring-summer vegetation permitted the increase up to 6.73-7.40 t/ha and 6.90-7.23 t/ha, respectively. In these years, considerably higher were the gross operating profit values and profitability values both in the control group and in the variations with nitrogen feedings as compared against 2012-year data.

Moreover, it should be mentioned that in 2013 and 2014 there were obtained the highest net income values from growing winter wheat after spring barley. This result was achieved with $N_{60}P_{60}K_{30}$ as a background and the plants were given the supplement of nitrogen fertilizers (ammonium nitrate) in two steps: N_{30} to the thawing frozen ground and N_{30} locally at the completion of tillering phase. After black fallow (on the background of $P_{60}K_{30}$), the values of this indicator were the highest in such fertilizering variations: N_{30-60} (ammonium nitrate) or N_{30} of UAN-32 at the end of plant tillering. The lowest results were attributed to those fertilizering variations where carbamide supplement was employed to the plants at their stage of heading and this did not allow the sharp increase in the yeild.

Quite unfavorable were also the weather conditions in autumn 2015. In spite of a rather lengthy period of autumn vegetation (65 days) and the sufficient sum of effective temperatures (294.1°C), the rainfall amount in this season was only 41.7 mm that is not enough for growing timely winter wheat seedlings after non-fallow previous crops, and, in particular after spring barley. During the winter period, the winter wheat seeds sown after stubble previous crop were at the stage of swelling and subsequent germination, while the sustainable seedlings appeared only in February at thawing periods. However, the specific character of 2015/2016 growing season was very good hydro and thermal conditions right after quite early spring vegetation recovery (1 March): they permitted the winter wheat plants to root well, to develop the sufficient leaf surfaces and above-ground vegetative masses and to produce rather good grain yield even after conventionally disadvantageous non-fallow previous crops.

The weather conditions of the following 2016/2017 and 2017/2018 growing seasons showed their specific character but in general were encouraging for the winter wheat grain yield, including the cases of sowing after spring barley. 2016 autumn period was rather cold but wet, while that in 2017 was on the contrary warm with the insufficient rainfall.

Should be noted that it was cold and frosty at nights but very wet in April in 2016/2017 growing season. In May, the weather was changeable with late frost. In spite of the mentioned, the state of the wheat improved at the end of May. In 2017/2018 growing season, the plants recovered their vegetation on 31 March, at the timing close to the climatic norm. March in 2018 was abnormally wet, the amount of the snow and rain precipitations totaled 145 mm, that was 4 times higher than the average values observed during the many years. In April, May and June, high air temperatures predominated, but the shortage of rainfall was also registered. In general, the moisturizing conditions in 2016-2018 were favorable during the spring vegetation of winter wheat, but they were unstable per months and wheat development stages.

The notable shortage of rainfall and insufficient sum value of the effective temperatures were also the characteristics of 2021/2022 autumn vegetation. This especially had the impact on winter wheat sown after non-fallow previous crops. However, moisture availability in April was considerably higher than the meteorological norms of the region and this contributed to the improvement in poorly developed wheat seedlings and enhanced their potential to sufficient productivity.

The research results point to the fact that 2016-2018 yields of winter wheat sown after spring barley depended on the winter wheat varieties and the fertilizing variation applied. The highest yield was observed with Pylypivka variety, strong in its grain quality, then followed the yield value obtained with Kokhanka variety. The variety of Misia Odeska in the above-mentioned years was inferior in the yield to others in all fertilizing variants of the research (Table 3).

Table 3. The economic efficiency of nitrogen feedings for winter wheat growing after spring barley, 2016-2018

Nitrogen feedings	Yield, t/ha	Grain class (DSTU 3768-2019)	Net income, UAH/ha	Profitability, %
Pylypivka				
No feeding - control (background $N_{60}P_{60}K_{30}$)	4.46	4	14,269	126.4
N_{30} in early spring on freeze-thawed soil	5.08	3	18,337	151.0
N_{60} in early spring on freeze-thawed soil	5.47	3	19,949	155.0
N_{30} at the end of plant tillering	4.97	3	17,613	144.3
N_{30} in early spring on freeze-thawed soil + N_{30} at the end of plant tillering	5.32	3	18,993	146.9
N_{60} at the end of plant tillering	5.25	3	18,564	143.5
N_{60} in early spring on freeze-thawed soil + N_{30} at the end of plant tillering	5.62	2	21,347	156.9
Kokhanka				
No feeding - control (background $N_{60}P_{60}K_{30}$)	4.26	4	13,139	116.6
N_{30} in early spring on freeze-thawed soil	4.91	3	17,319	142.6
N_{60} in early spring on freeze-thawed soil	5.31	3	18,986	147.5
N_{30} at the end of plant tillering	4.74	3	16,264	133.6
N_{30} in early spring on freeze-thawed soil + N_{30} at the end of plant tillering	5.16	3	18,030	139.4
N_{60} at the end of plant tillering	5.08	3	17,545	135.6
N_{60} in early spring on freeze-thawed soil + N_{30} at the end of plant tillering	5.46	3	19,147	140.7
Misia Odeska				
No feeding - control (background $N_{60}P_{60}K_{30}$)	3.88	4	11,122	100.1
N_{30} in early spring on freeze-thawed soil	4.45	4	13,558	113.5
N_{60} in early spring on freeze-thawed soil	4.78	3	16,041	126.9
N_{30} at the end of plant tillering	4.24	4	12,340	103.2
N_{30} in early spring on freeze-thawed soil + N_{30} at the end of plant tillering	4.60	3	14,921	117.7
N_{60} at the end of plant tillering	4.54	3	14,546	114.6
N_{60} in early spring on freeze-thawed soil + N_{30} at the end of plant tillering	5.03	3	16,752	124.8

The nitrogen supplements of ammonium nitrate depended on the doses and the time of their application, they contributed to the Pylypivka yield increase, namely from 4.46 to 4.97-5.62 t/ha; Kokhanka variety - from 4.26 to 4.74-5.46 t/ha, while Misia Odeska - from 3.88 to 4.24-5.03 t/ha. The highest increases in grain yield compared against the control group were ensured by applying nitrogen fertilizer of N_{60} on freeze-thawed soil in two stages: N_{60} on freeze-thawed soil + N_{30} locally at the end of plant tillering.

Further, as compared with the control group, the winter wheat grain quality was better in the variations where nitrogen feedings were applied and mainly those of the 4th and the 3^{d} class. With Pylypivka variety, the grain of the 2nd class was obtained. Furthermore, according to the Ukrainian standard on soft wheat (DSTU 3768-2019) the 1st class of wheat is the highest grade, followed in quality degrading by the 2nd and the 3d class, and the 4th is the lowest class. Therefore, the 1st class grain is the most expensive for selling, while the 4th class is the cheapest.

When determining the economic efficiency for winter wheat growing (as we mentioned before) we took into account both the yield and the grain quality. The costs of the mineral fertilizers in the years of long-term studies are among the main constituents of the operational costs of winter wheat growing. The expenses spent at various agricultural practices were also considered in the estimation: they were the introduction of fertilizers, preventive measures against diseases, pests and weeds, crop gathering, crop delivery to the elevators and others.

The analysis on the experimental data has revealed that the highest yields and net incomes of winter wheat sown after spring barley in 2016-2018 were obtained in variations where along with $N_{60}P_{60}K_{30}$, the ammonium nitrate (N_{60}) supplement was introduced on freeze-thawed soil or in two stages: N_{60} on freeze-thawed soil and N_{30} locally at plant tillering phase. With the described fertilizing, Pylypivka variety produced 5.47 t/ha and 5.62 t/ha, Kokhanka variety - 5.31 t/ha and 5.46 t/ha, the variety of Misia Odeska - 4.78 t/ha and 5.03 t/ha.

The net income with Pylypivka variety compared with the other varieties under study was the highest and was attributed to above-mentioned supplement variations: 10,049 UAH/ha and 21,347 UAH/ha, Kokhanka variety - 18,986 UAH/ha and 19,147 UAH/ha, Misia Odeska - 16,041 UAH/ha and 16,752 UAH/ha.

Wheat grain growth profitability in control groups (without nitrogen feedings during the period of spring vegetation) was the lowest: within 100.1-126.4 % depending on the variety.

The introduction of nitrogen feedings facilitated the increase in this value: 17.1-30.5 % higher profitability with Pylypivka, 17.0-30.9 % - Kokhanka, 13.4-24.7 % - Misia Odeska.

CONCLUSIONS

Based on the long-term research to have been carried out under conditions of Ukrainian Northern Steppe, the specific character of winter wheat growing after spring barley has been described and it has been determined that feeding with nitrogen fertilizers does not ensure higher productivity of the plants if there was drought at their spring-summer vegetation. In a number of cases, winter wheat growing in such years at the plots where nitrogen feedings were introduced into the soil was unprofitable, while the control variants (without application of nitrogen fertilizers) provided the highest net income and the profitability.

When moisturizing was sufficient in the described period of time, the mineral fertilizers dissolved within the ground and were better consumed by the plants, the yield and the economic efficiency of wheat growing increased. The highest values of the yield and the net income were obtained from winter wheat growing after stubble previous crop on average in 2016-2018 with those variations, where on the background of $N_{60}P_{60}K_{30}$ we applied ammonium nitrate (N_{60}) supplement introduced on freeze-thawed soil or in two stages: N_{60} on freeze-thawed soil and N_{30} locally at plant tillering phase. These data were varying respectively to the fertilizing variations within 4.78-5.47 t/ha and 16,041-19,949 UAH/ha, 5.03-5.62 t/ha and 16,752-21,347 UAH/ha. Among the varieties of Kokhanka, Misia Odeska and Pylypivka, the maximal economic efficiency was registered with the variety of Pylypivka, which had the highest yield and the best grain quality.

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STUDY OF AGRONOMIC CHARACTERISTICS OF SOME CORN LINES CREATED AT SCDA LOVRIN

Marinel Nicolae HORABLAGA[1, 2], Codruța CHIȘ[1], Ștefan Laurențiu BĂTRÎNA[1], Ilinca Merima IMBREA[1], Adina HORABLAGA[1], Florin IMBREA[1]

[1]University of Life Sciences "King Mihai I" from Timisoara, 119 Calea Aradului Street, Timisoara, Romania
[2]Lovrin Agricultural Research and Development Station, 200 Principala Street, Lovrin, Romania

Corresponding author email: codrutachis@gmail.com

Abstract

The aim of this research was to analyse the agronomic characteristics of a maize variety consisting of 20 inbred lines, which are used as germplasm sources for hybrid maize production at SCDA Lovrin. The statistical analysis and interpretation of the results were carried out using IBM SPSS software. Descriptive statistics, including mean, median, standard deviation, minimum, and maximum values, were calculated. To evaluate the significance of the differences, ANOVA and Duncan's multiple range tests, as well as Kruskal-Wallis and Mann-Whitney U tests, were performed. Pearson correlation coefficients and regression lines were determined to study the relationships between variables. The results indicate significant differences between the studied traits, such as leaf color, leaf orientation, anther color (tassel), silk color, ear position (degree), total number of leaves, number of leaves up to the main ear, and number of tassel branches. Additionally, a linear correlation was observed between the number of growing degree days (GDD) to flowering/silking and the total accumulated GDD until flowering/silking.

***Key words**: maize inbred line, agronomical traits.*

INTRODUCTION

Maize (*Zea mays* L.) is an important and versatile crop, serving as a primary source of food for both humans and animals and as a vital raw material for various industrial sectors (Troyer, 2000). With its widespread use, maize has become the third most extensively cultivated crop globally. In Romania, maize is a major crop, covering an estimated area of approximately 3 million hectares annually (Sarca, 2004).
The current climate change scenario has posed significant challenges for maize growers, particularly with regard to water and heat stress (Nagy, 2004; Musteata et al., 2005). To address these challenges, maize breeders must develop new hybrids with improved stress tolerance (Roman et al., 1973; Troyer, 1999; Suteu et al., 2013). Therefore, studies that analyse the agronomic traits of maize inbred lines that serve as the foundation for developing new hybrids are of great importance (Has, 1999; Has et al., 1999, Troyer, 1999).
Moreover, the importance of identifying and developing maize germplasm adapted to specific environmental conditions has become increasingly vital, particularly in areas where there are recurrent climatic disturbances (Cabulea et al., 1975; Roman, 1976; Duvick, 1984). Therefore, the identification of new maize hybrids tolerant to such conditions will be critical in maintaining and increasing maize productivity in these areas (Grecu, 1962; Grecu and Has, 2001; Musteata et al., 2005).

MATERIALS AND METHODS

The material subjected to analysis consisted of 20 inbred lines from SCDA Lovrin, used as germplasm source in the breeding process. The experiment was conducted using a comparative culture method, on a cambic chernozem soil with a shallow water table, wet and weakly saline (under 100 cm), with moderate alkalization, low decarbonatization, on sandy loam soils with sand parent material and water table depth at 2-5 m. Analytical data of the soil profile from Lovrin Agricultural Research and Development Station, where the research was conducted, are presented in Table 1.

Figure 1 displays the average decadal, monthly, and annual temperatures, the multi-year averages, as well as the decadal and monthly precipitation amounts during the experimental period.

Regarding the multi-year averages for precipitation accumulated during the vegetation period, a deficit of 44.6 mm was observed in 2019, a deficit of 49.2 mm was observed in 2020, while an excess of 78 mm/ha was recorded in 2021. Notably, in 2021, which was the most favourable year for maize cultivation, the amount of precipitation accumulated in June exceeded the monthly average by 26.9 mm/ha, and in August, the accumulated precipitation exceeded the multi-year monthly average by 10.9 mm/ha. It is important to highlight that the annual average temperature exceeded the multi-year average by 1.7°C in all three experimental years, by 1.6°C in 2020, and by 1.4°C in 2021.

Table 1. The main features of cambic chernozem, phreatic moist, in Lovrin

Characteristics	Ap	Am	A/C	Ccaac	C/CaGoac
Depths (cm)	0-26	26.47	47-49	79-123	123-200
Coarse sand (2.0-0.2 mm)%	1	0.9	0.5	0.6	0.3
Fine sand (0.2-0.02 mm)%	34.3	36.4	35.2	38.5	24.7
Dust (0.02-0.002 mm)%	27.7	26.5	28.9	31.3	42.6
Clay (< 0.002 mm)%	37	36.2	35.4	29.6	32.4
Physical clay (< 0.01 mm)%	51.8	51.7	51	46.8	57
Specific density(D g/cm^3)		2.55	2.56	2.63	
Apparent density (DA g/cm^3)		1.4	1.33	1.27	
Total porosity (PT %)		45	48	50	
Aeration porosity (PA %)		9	13	16	
Coefficient of higroscopicity (CH%)		8	7.8	7	
Coefficient of withering (CO %)		12	11.7	10.5	
Field capacity (CCA)		26	26	25.4	
Useful water capacity(CU%)		14	14.3	14.8	
Total capacity for water(CT %)		32.2	36.1	37.9	
PH in H_2O	6.9	7.2	8.45	9.4	9.45
Carbonates ($CaCO_3$%)		0.4	9.8	19.3	16
Hydraulic conductivity(K = mm/h)		11.9	10.3	12.8	
Humus (%)	3.47	3.28	2.73		
Nr. of bacteria mil/100 g dry soil	772				
C: N	13.7	13.9	13.7		
Total nitrogen (%)	0.171	0.159	0.12		
Mobile phosphorus (ppm)	75.7	31.7	8.7		
Mobile potassium (ppm)	205	202	163		
Bases of exchange (SB me la 100 g sol)	27.6	27.6	20.3		
Hydrogen exchange (Sh me la 100 g sol)	4.35				
Cation exchange cap. (T me la 100 g sol)	32	27.6	21.9		
Degree of saturation in bases (V%)	86.4	100	100	100	100
EC mmho/cm	0.78	0.57	1.68		
Na^+ (me per 100 g soil)	0.04	0.1	0.66		
Na^+ exchangeable (% of T)	0.6	0.5	3.5	13.1	13

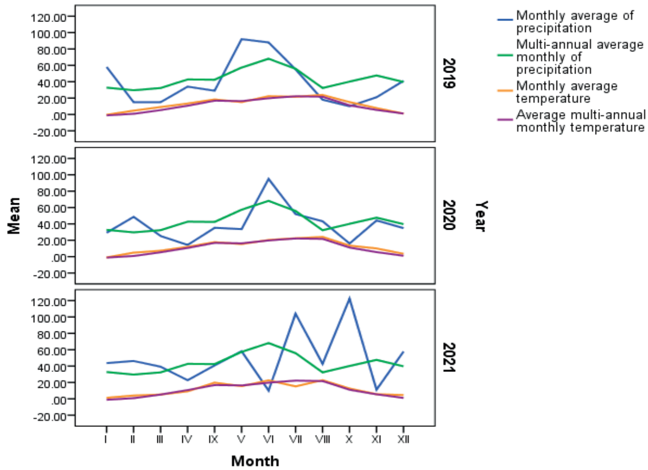

Figure 1. Temperature and precipitation evolution in 2019-2021 period

RESULTS AND DISCUSSIONS

The statistical analysis of the data was performed using IBM SPSS statistical software. Descriptive characteristics such as mean, median, standard deviation, minimum and maximum values were calculated.

To analyse significant differences, ANOVA and Duncan tests, as well as Kruskal-Wallis and Mann-Whitney tests were applied. For the study of relationships between variables, Pearson correlation coefficients and regression lines were determined

Regarding the leaf color, it was observed that 40% of the analysed lines (5013, 5027, 5071, 5104, 5174, 5178, 5186, and 5190) exhibit a light green colour, while 60% of the lines (5001, 5006, 5008, 5009, 5059, 5075, 50164, 5167, 5170, 5171, 5181, and 5216) show a dark green colour.

Analysing the leaf habit of the 20 selected lines, it was found that 90% of the lines have a semi-erect habit. Only two lines (5059 and 5216) exhibit an erect leaf habit, indicating their potential contribution to the creation of hybrids capable of withstanding higher densities and displaying increased resistance to drought due to a reduction in evapotranspiration surface area.

The positions of the cobs varied between 30° and 45°, being distributed as follows: 20% of the lines at 30°, 25% of the lines at 35°, 10% at 40°, and 45% of the lines at 45°.

The total number of leaves for the lines in the study ranged from a minimum of 9 leaves to a maximum of 15 leaves per line, with a minimum percentage of 5% for the lines with 9 and 10 leaves, reaching a maximum percentage of 25% for the lines with 11 and 12 leaves per line.

The minimum number of leaves to the main ear was for 5% of the lines, the maximum of 9 leaves was found in 10% of the lines, while the maximum percentage of 45% of the lines had 6 leaves to the main ear.

The average number of total leaves per plant is 12.3 ± 1.657, with a minimum of 9 leaves and a maximum of 15 leaves, while the average number of leaves to the main ear is 6.55 ± 1.316, with a minimum of 4 and a maximum of 9 leaves (Figure 2).

The number of cobs per plant was one cobin 85% of the lines, two cobs in 10% of the lines (5071, 5178), and only 5% of the lines had three cobs per plant (5014).

The total plant height varied from 92 cm in line 5174 to a maximum of 163 cm in line 5075. The average height of the experiment was 130 cm (Figure 3).

By applying ANOVA, significant differences were observed between the studied lines in terms of total plant height (F=9.724, p=0.000). Lines 5027, 5059, 5075, and 5167 showed significant positive differences compared to the field average.

Based on the Duncan test, significant differences were determined between all these lines (Table 2).

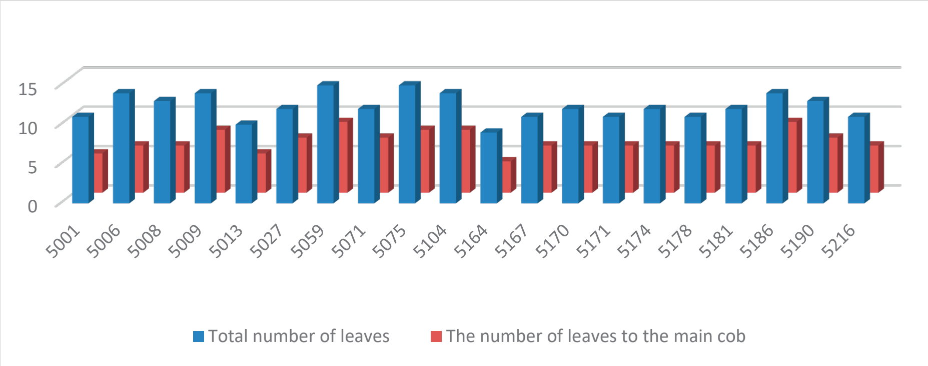

Figure 2. Total number of leaves, respectively the number of leaves to the main cob

Table 2. Numeric traits associated to plant height

Line	Mean (m)	SD	95% Confidence Interval for Mean		Min.	Max.	Diff.	Semnif.	p	Duncan test
			Lower Bound	Upper Bound						
Field average	1.3066	0.0447	1.2746	1.3385	1.23	1.37	0	-	-	defg
5001	1.262	0.1834	1.1308	1.3932	0.98	1.52	-0.0445	ns	0.3947	bcdef
5006	1.335	0.0756	1.2809	1.3891	1.22	1.47	0.0285	ns	0.5865	efgh
5008	1.39	0.1222	1.3026	1.4774	1.25	1.58	0.0835	ns	0.1117	ghij
5009	1.237	0.0721	1.1854	1.2886	1.15	1.37	-0.0695	ns	0.1845	bcdef
5013	1.346	0.134	1.2501	1.4419	1.1	1.57	0.0395	ns	0.4509	fghi
5027	1.452	0.0771	1.3968	1.5072	1.27	1.53	0.1455	**	0.0059	ijk
5059	1.426	0.0662	1.3786	1.4734	1.33	1.52	0.1195	*	0.0233	hijk
5071	1.204	0.0949	1.1361	1.2719	1.04	1.34	-0.1026	ns	0.051	bcd
5075	1.521	0.0684	1.4721	1.5699	1.45	1.63	0.2145	***	0.0001	1k
5104	1.388	0.1336	1.2925	1.4835	1.09	1.62	0.0815	ns	0.1205	ghij
5164	1.158	0.1657	1.0395	1.2765	0.97	1.37	-0.1486	00	0.0049	ab
5167	1.498	0.1603	1.3833	1.6127	1.25	1.74	0.1915	***	0.0003	jk
5170	1.31	0.1599	1.1956	1.4244	1.14	1.61	0.0035	ns	0.9474	defgh
5171	1.321	0.1436	1.2182	1.4238	1.04	1.45	0.0145	ns	0.7823	defgh
5174	1.059	0.0937	0.9919	1.1261	0.92	1.29	-0.2476	000	0.000	a
5178	1.235	0.082	1.1764	1.2936	1.12	1.33	-0.0715	ns	0.1723	bcdef
5181	1.174	0.0906	1.1092	1.2388	0.99	1.34	-0.1326	0	0.0119	bc
5186	1.292	0.1062	1.216	1.368	1.14	1.52	-0.0146	ns	0.7808	cdefg
5190	1.221	0.086	1.1595	1.2825	1.1	1.35	-0.0855	ns	0.103	bcde
5216	1.302	0.1527	1.1928	1.4112	1.11	1.53	-0.0045	ns	0.9307	defg

ns= Not significant. * The mean difference positive and is significant at the 0.05 level. ** The mean difference positive and is significant at the 0.01 level. *** The mean difference positive and is significant at the 0.001 level. 0 - The mean difference negative and is significant at the 0.05 level. 00 - The mean difference negative and is significant at the 0.01 level. 000 - The mean difference negative and is significant at the 0.001 level.

The data regarding the height of the main cob insertion point shows that it varied between 25 cm in line 5164 and 80 cm in line 5104. Lines 5008 and 5186 had a cob insertion height of 75 cm (Figure 3).

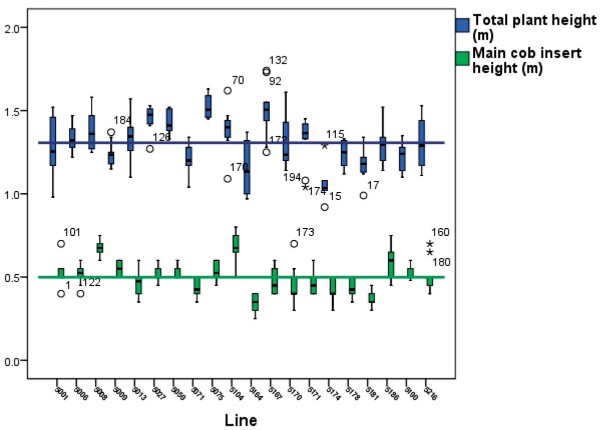

Figure 3. Box plots associated with the characteristics "Total plant height" and "Height of main cob insertion"

The lines differ significantly in terms of the variable "height of the main cob insertion point" ($F = 16.290$, $p = 0.000$). It can be observed that lines 5008, 5014, and 5186 are the ones that showed significant positive differences compared to the field average (Table 3). Based on the Duncan test, significant differences were determined between all of these lines (Table 3).

Table 3. Numeric traits associated to height of the main cob insertion point

Line	**Mean**	**SD**	**95% Confidence Interval for Mean**		**Min.**	**Max.**	**Diff.**	**Semnif.**	**p**	**Duncan test**
			Lower Bound	**Upper Bound**						
Field media	0.4993	0.0137	0.4895	0.5090	0.47	0.52	0	ns		**defg**
5001	0.5200	0.0753	0.4661	0.5739	0.4	0.7	0.021	ns	0.4784	bcde
5006	0.5200	0.0633	0.4748	0.5652	0.4	0.6	0.021	ns	0.4784	efgh
5008	0.6750	0.0425	0.6446	0.7054	0.6	0.75	0.1758	***	0.000	fgh
5009	0.5500	0.0408	0.5208	0.5792	0.5	0.6	0.051	ns	0.084	bcde
5013	0.4600	0.0738	0.4072	0.5128	0.35	0.6	-0.039	ns	0.1807	efgh
5027	0.5250	0.0486	0.4902	0.5598	0.45	0.6	0.026	ns	0.3792	h
5059	0.5250	0.0425	0.4946	0.5554	0.5	0.6	0.026	ns	0.3792	gh
5071	0.4350	0.0530	0.3971	0.4729	0.35	0.5	-0.0643	0	0.0291	bc
5075	0.5350	0.0530	0.4971	0.5729	0.45	0.6	0.036	ns	0.2226	i
5104	0.6750	0.0858	0.6136	0.7364	0.5	0.8	0.1758	***	0.000	efgh
5164	0.3450	0.0550	0.3056	0.3844	0.25	0.4	-0.1543	000	0.000	a
5167	0.4750	0.0825	0.4160	0.5340	0.4	0.6	-0.024	ns	0.4076	i
5170	0.4500	0.1106	0.3709	0.5291	0.3	0.7	-0.049	ns	0.0935	defgh
5171	0.4600	0.0615	0.4160	0.5040	0.4	0.6	-0.039	ns	0.1807	efgh
5174	0.4200	0.0675	0.3717	0.4683	0.3	0.5	-0.0793	00	0.0073	a
5178	0.3900	0.0928	0.3917	0.1172	0.4	0.35	-0.0643	0	0.0291	bcd
5181	0.3620	0.0434	0.3309	0.3931	0.3	0.45	-0.1373	000	0.000	b
5186	0.5850	0.0944	0.5175	0.6525	0.45	0.75	0.0858	*	0.0037	bcdef
5190	0.5180	0.0368	0.4917	0.5443	0.48	0.6	0.019	ns	0.5218	bc
5216	0.5150	0.0914	0.4496	0.5804	0.4	0.7	0.016	ns	0.5904	cdefg

ns = Not significant. * The mean difference positive and is significant at the 0.05 level. ** The mean difference positive and is significant at the 0.01 level. *** The mean difference positive and is significant at the 0.001 level. 0 - The mean difference negative and is significant at the 0.05 level. 00 -The mean difference negative and is significant at the 0.01 level. 000 - The mean difference negative and is significant at the 0.001 level.

The length of the leaves of the main cob (Figure 4) ranged from 42 cm in line 5181 to 75 cm in line 5059, with line 5059 having the highest average length of leaves (Figure 5). Analysis of the length of leaves of the main cob shows significant differences between the analysed lines (F = 18.504, p = 0.000) and compared to the field average. Lines 5008, 5027, 5059, 5075, 5014, and 5017 differ significantly in a positive sense compared to the field average (Table 4).

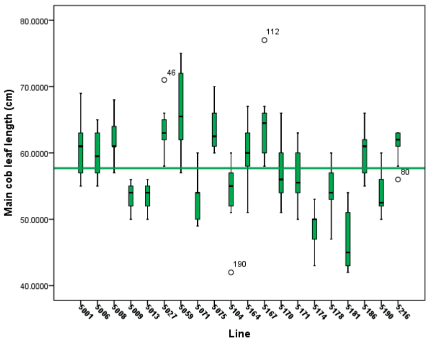

Figure 4. Boxplot Diagram associated with the characteristic of the main cob leaf length

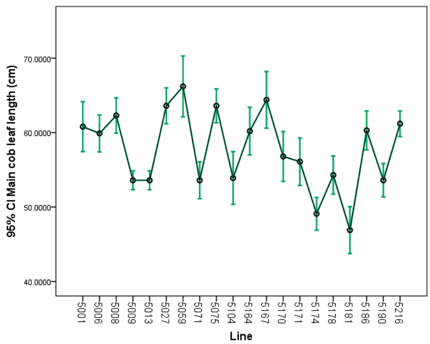

Figure 5. Averages and confidence intervals for variable Cob leaf length main cob

Table 4. Numeric characteristics associated with the main cob leaf length

Line	Mean (cm)	SD	95% Confidence Interval for Mean		Min.	Max.	Diff.	Semnif.	P	Duncan test
			Lower Bound	Upper Bound						
Field mean	57.7	0.8062	57.123	58.277	56.4	59.2				**cde**
5001	60.8	4.6857	57.448	64.152	55	69	3.1	ns	0.0698	bc
5006	59.9	3.4785	57.412	62.388	55	65	2.2	ns	0.1972	def
5008	62.3	3.3015	59.938	64.662	57	68	4.6	**	0.0074	fg
5009	53.6	1.7764	52.329	54.871	50	56	-4.1	0	0.0168	b
5013	53.6	1.7764	52.329	54.871	50	56	-4.1	0	0.0168	efg
5027	63.6	3.3731	61.187	66.013	58	71	5.9	***	0.0006	fgh
5059	66.2	5.7116	62.114	70.286	57	75	8.5	***	0.000	fg
5071	53.6	3.4705	51.117	56.083	49	60	-4.1	0	0.0168	b
5075	63.6	3.1693	61.333	65.867	60	70	5.9	***	0.0006	h
5104	53.9	4.9542	50.356	57.444	42	60	-3.8	*	0.0266	efg
5164	60.2	4.492	56.987	63.413	51	67	2.5	ns	0.1431	a
5167	64.4	5.3166	60.597	68.203	58	77	6.7	***	0.0001	gh
5170	56.8	4.6857	53.448	60.152	51	66	-0.9	ns	0.5972	def
5171	56.1	4.4585	52.911	59.289	50	63	-1.6	ns	0.3479	def
5174	49.1	3.0714	46.903	51.297	43	53	-8.6	000	0.000	a
5178	54.3	3.5917	51.731	56.869	47	60	-3.4	0	0.0469	b
5181	46.9	4.4083	43.746	50.054	42	54	-10.8	000	0.000	b
5186	60.3	3.653	57.687	62.913	55	66	2.6	ns	0.1279	bc
5190	53.6	3.134	51.358	55.842	50	60	-4.1	0	0.0168	b
5216	61.2	2.3944	59.487	62.913	56	63	3.5	0	0.0409	bcd

ns = Not significant. * The mean difference positive and is significant at the 0.05 level. ** The mean difference positive and is significant at the 0.01 level. *** The mean difference positive and is significant at the 0.001 level. 0 - The mean difference negative and is significant at the 0.05 level. 00 -The mean difference negative and is significant at the 0.01 level. 000 - The mean difference negative and is significant at the 0.001 level.

The diagram referring to the width of the main cob leaf, presented in Figure 6, recorded values between 5 cm and 9 cm. The average of the measurements was 6.85 cm. The width of the main cob leaf differs significantly from both the field average and the lines considered in the study (F = 15.490, p = 0.000). Lines 5001, 5006, 5013, 5075, and 5170 show significant positive differences compared to the field average (Table 5).

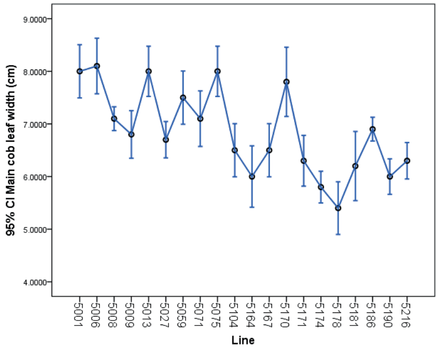

Figure 6. Mean and 95% CI for the main cob leaf width

Table 5. Numeric characteristics associated with the width of the cob leaf main

Line	Mean	Std. Deviation	95% Confidence Interval for Mean		Min.	Max.	Diff.	Semnif.	p	Duncan Test
			Lower Bound	Upper Bound						
Field mean	6.85	0.143	6.75	6.95	7	9				**cde**
5001	8	0.707	7.49	8.51	7	9	1.15	***	0.0001	bc
5006	8.1	0.738	7.57	8.63	7	9	1.25	***	0.000	def
5008	7.1	0.316	6.87	7.33	7	8	0.25	ns	0.388	fg
5009	6.8	0.632	6.35	7.25	6	8	-0.05	ns	0.8628	b
5013	8	0.667	7.52	8.48	7	9	1.15	***	0.0001	efg
5027	6.7	0.483	6.35	7.05	6	7	-0.15	ns	0.6042	fgh
5059	7.5	0.707	6.99	8.01	6	8	0.65	*	0.0256	fg
5071	7.1	0.738	6.57	7.63	6	8	0.25	ns	0.388	b
5075	8	0.667	7.52	8.48	7	9	1.15	***	0.0001	h
5104	6.5	0.707	5.99	7.01	5	7	-0.35	ns	0.2273	efg
5164	6	0.816	5.42	6.58	5	7	-0.85	00	0.0037	a
5167	6.5	0.707	5.99	7.01	6	8	-0.35	ns	0.2273	gh
5170	7.8	0.919	7.14	8.46	6	9	0.95	**	0.0012	def
5171	6.3	0.675	5.82	6.78	5	7	-0.55	ns	0.0585	def
5174	5.8	0.422	5.5	6.1	5	6	-1.05	000	0.0004	a
5178	5.4	0.699	4.9	5.9	5	7	-1.45	000	0.000	b
5181	6.2	0.919	5.54	6.86	5	7	-0.65	0	0.0256	b
5186	6.9	0.316	6.67	7.13	6	7	0.05	ns	0.8628	bc
5190	6	0.471	5.66	6.34	5	7	-0.85	00	0.0037	b
5216	6.3	0.483	5.95	6.65	6	7	-0.55	ns	0.0585	bcd

ns = Not significant. * The mean difference positive and is significant at the 0.05 level. ** The mean difference positive and is significant at the 0.01 level. *** The mean difference positive and is significant at the 0.001 level. 0 - The mean difference negative and is significant at the 0.05 level. 00 - The mean difference negative and is significant at the 0.01 level. 000 - The mean difference negative and is significant at the 0.001 level.

The analysis shows that there are significant differences in the number of branches on the panicle between the studied lines (χ^2 = 46.924, p = 0.001, Kruskal Wallis test). By applying the Mann-Whitney test, significant differences were found compared to the field mean, specifically for lines 5006 (p = 0.0462), 508 (p = 0.0463), 5059 (p = 0.0461), 5216 (p = 0.0463).
The total plant height is moderately positively correlated with the height of the insertion of the main cob (R = 0.554, p = 0.000), positively correlated with the length of the main cob leaf (R = 0.437, p = 0.000), and weakly positively correlated with the width of the main cob leaf (R = 0.264, p = 0.000) (Figure 7).
The height of the insertion of the cob is weakly positively correlated with the length of the cob leaf (R = 0.208, p = 0.000) and weakly positively correlated with the width of the main cob leaf (R = 0.165, p = 0.020). There is a weak positive correlation between the length and width of the main cob leaf (R = 0.398, p = 0.000) (Figure 7).

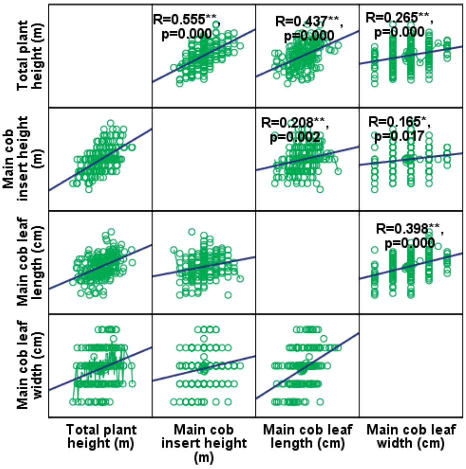

Figure 7. Matrix of correlations between variables
**Correlation is significant at the 0.01 level (2-tailed)
*Correlation is significant at the 0.05 level (2-tailed)

The average number of days until emergence is 10.2 days with a standard deviation of 1.19 days, lines 5006, 5013, 5164, 5167, 5178, 5181, 5216 being those that sprung up after a minimum of 9 days, and the last lines that have sprung up are 5008, 5059, 5075, 5190 after a maximum number of days of 12, (Figure 8, Table 6). The median number of days until flowering is Me=10 days so that 50% of the lines (10) bloomed before 10 days.

Table 6. Descriptive numeric characteristics associated with the number of days until emergence/flowering/sweeping

Descriptive numeric characteristics	Mean	Median	SD	Min.	Max.	Percentiles	
						25(Q_1)	75(Q_3)
Date to plant emergence	10.2	10	1.1964	9	12	9	11
Days to flowering	78.7	78	1.9221	77	82	77	80.75
Days to silk	81.45	80.5	2.0895	79	85	80	83.75

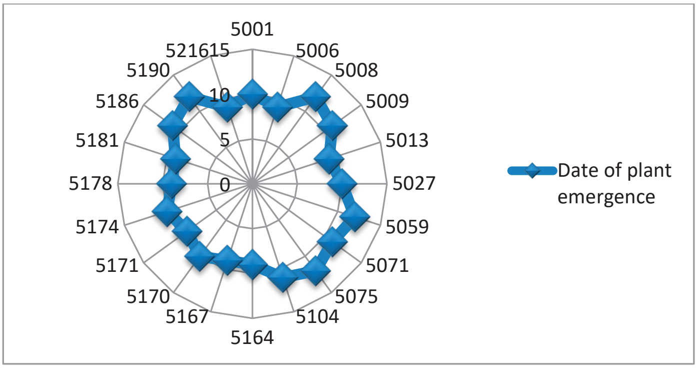

Figure 8. Number of days until sunrise by lines

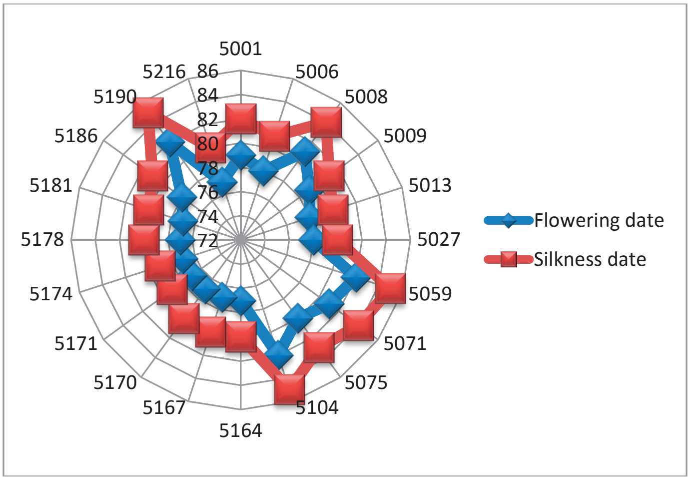

Figure 9. Number of days until flowering/silking by lines

Lines 5164, 5167, 5170, 5171, 5174, 5178, 5181, 5216, 5006, 5013, 5027 are those with a lower number of days until flowering/ sweeping (Figure 9).

The analysis of the correlation between the number of days to flowering and the number of days to silking shows a significant positive direct linear correlation. The number of days to flowering (NDF) is highly positively correlated with the number of days to silking (NDS), with an R value of 0.979 (p = 0.0000, correlation is significant at the 0.01 level).

The linear model was a good fit for the data (p = 0.000, F = 413.340), and the regression line obtained was y = 1.0641 x-2.2948 (t = 20.331, p = 0.000) (Figure 10). The mean of the accumulated thermal units until flowering was 17345.242 (degree Celsius)/hour, with a standard deviation of 692.2489 (degree Celsius) (Table 7).

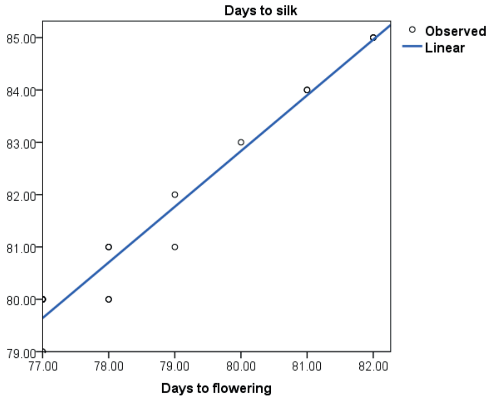

Figure 11. The regression line between the number of days to flowering (DF) and the number of days to silking (DS)

Table 7. Descriptive numeric characteristics associated with variables

Descriptive numeric characteristics	Mean	Median	SD	Min.	Max.
The sum of thermal degrees until flowering day(C)/Degrees hours	17345.242	17117.675	692.2489	16672.54	18567.72
The sum of thermal degrees until flowering day(C)/Degrees days	716.0715	706.27	29.56702	688.72	767.8
The sum of thermal degrees to silky day (C)/Degrees hours	18455.257	18164.175	635.14167	17504.55	19534.27
The sum of thermal degrees to silky day (C)/Degrees days	762.3185	749.93	27.11887	723.38	808.08

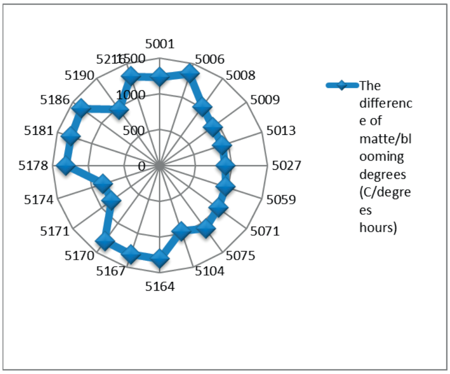

Figure 12. The difference of matte/blooming degrees (C/degrees hours)

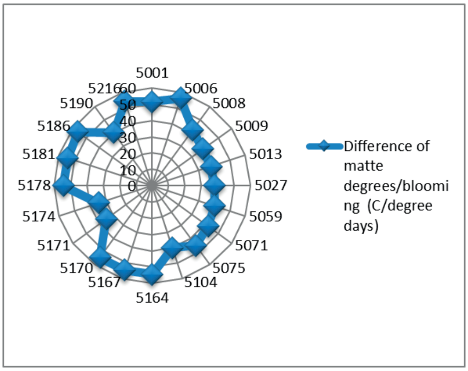

Figure 13. Difference of matte degrees/blooming (C/degrees days)

The sum of thermal degrees until the day of flowering (in Celsius) and degree hours show a direct linear correlation that is statistically significant with Days to flowering, with an R value of 0.975 (p = 0.000, indicating that the correlation is significant at the 0.01 level). The linear model fits the data well (F = 1643.611, p = 0.000), resulting in a regression line of y = 0.003x+30.799 (t = 40.541, p = 0.000) (Figure 14).

Similarly, the variables, the sum of thermal degrees until flowering day (in Celsius)/degree days and Days to flowering also show a significant direct correlation (R = 0.997, p = 0.000, indicating that the correlation is significant at the 0.01 level). The regression line obtained fits the data well (F = 2609.7, p = 0.000), resulting in y = 0.065x+32.307 (t = 51.085, p = 0.000) (Figure 15)

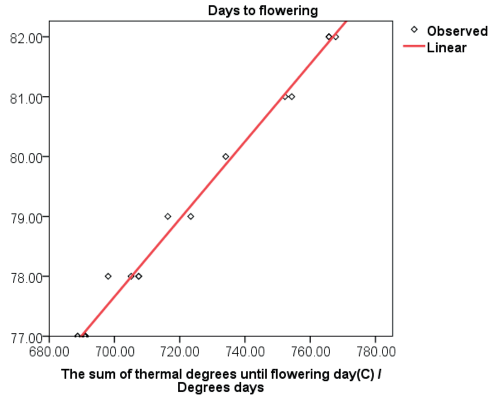

Figure 13. Regression line between The sum of thermal degrees until flowering day (C)/Degrees hours and the number of days to bloom (DF)

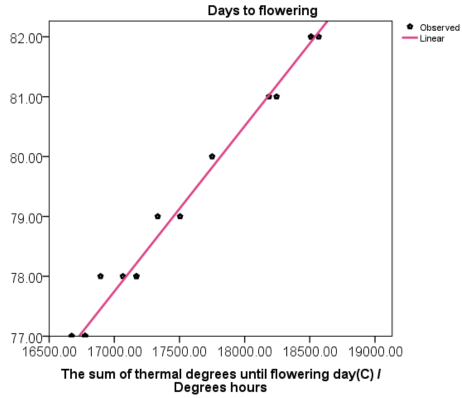

Figure 14. Regression line between The sum of thermal degrees until flowering day(C)/day hours and the number of days until flowering (DF)

The sum of thermal degrees until the day of flowering (in Celsius) and degree hours show a direct linear correlation that is statistically significant with Days to silk, with an R value of 0.977 (p = 0.000, indicating that the correlation is significant at the 0.01 level). The linear model fits the data well (F = 380.4917, p = 0.000), resulting in a regression line of y = 0.003x+30.292 (t = 19.506, p = 0.000) (Figure 15).

Similarly, the variables The sum of thermal degrees until flowering day (in Celsius)/degree days and Days to silk also show a significant direct correlation (R = 0.978, p = 0.000, indicating that the correlation is significant at the 0.01 level). The regression line obtained fits the data well (F = 393.3452, p = 0.000), resulting in y = 0.069x+31.966 (t = 51.085, p = 0.000) (Figure 17).

The sum of thermal degrees until the day of silk (in Celsius) and degree hours shows a direct linear correlation that is statistically significant with Days to silk, with an R value of 0.989 (p = 0.000, indicating that the correlation is significant at the 0.01 level). The linear model fits the data well (F = 824.3406, p = 0.000), resulting in a regression line of y = 0.003x+21.389 (t = 28.711, p = 0.000) (Figure 16).

Similarly, the variables The sum of thermal degrees until silk day (in Celsius)/degree days and Days to silk also show a significant direct correlation (R = 0.998, p = 0.000, indicating that the correlation is significant at the 0.01 level). The regression line obtained fits the data well (F = 1180.965, p = 0.000), resulting in y = 0.076x+23.158 (t = 34.365, p = 0.000) (Figure 18).

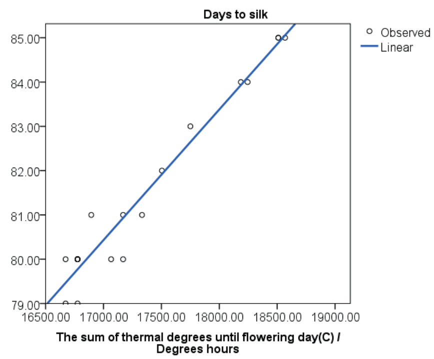

Figure 15. Regression line of the sum of thermal degrees until flowering day(C)/Degrees hours and days to silk

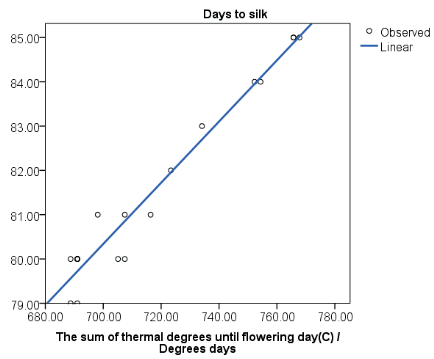

Figure 16. Regression line of the sum of thermal degrees until flowering day(C)/Degrees days and days to bloom (DF)

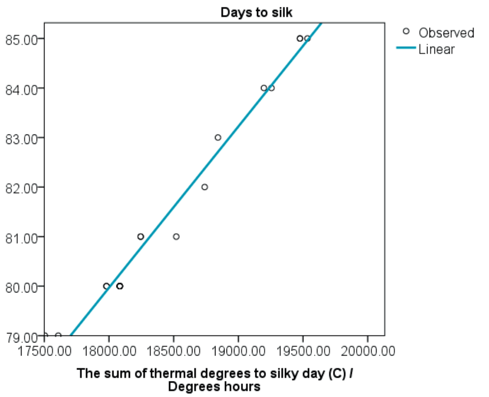

Figure 17. Regression line of the sum of thermal degrees until silky day(C)/Degrees hours and days to silk

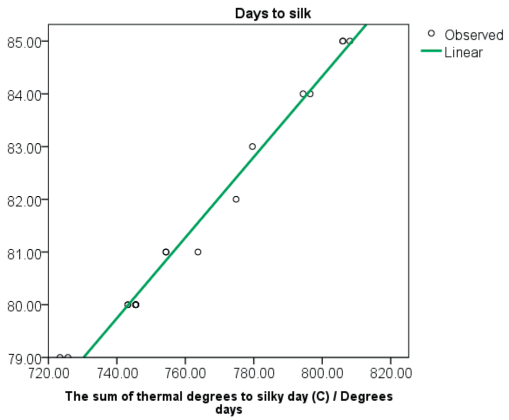

Figure 18. Regression line of the sum of thermal degrees until flowering day (C)/Degrees days and days to bloom

A regression model was also determined between the variable Number of days to silk (x), Panicle branches (y), and the variable Total number of leaves (z) (R = 0.704, F = 8.359, p = 0.003 < 0.05) using the equation: $z = 0.796x+0.315y+68.756$ (Figure 19).

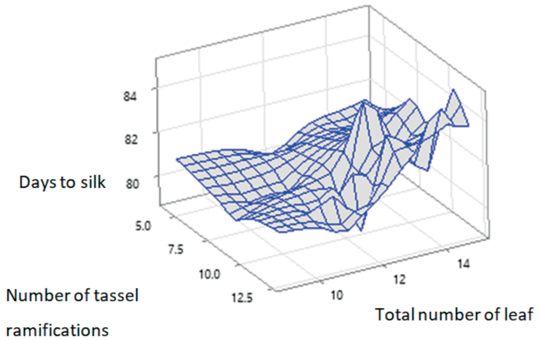

Figure 19. Surface plot of Day to silk vs. Total number of leaf and Number of tassel ramifications

CONCLUSIONS

The results obtained regarding the analysis of certain agronomic characteristics on 20 maize lines belonging to SCDA Lovrin confirm their exceptional value as a germplasm source for maize breeding and obtaining hybrids with valuable traits.

The average number of days to germination is 10.2 days with a standard deviation of 1.19 days, lines 5006, 5013, 5164, 5167, 5178, 5181, 5216 being those that germinated after a minimum of 9 days, while the last lines that germinated are 5008, 5059, 5075, 5190 after a maximum number of 12 days. The median of the number of days to flowering showed that 50% of the lines (10) flowered before 10 days.

The results regarding the number of days to flowering/silking, as well as the thermal requirement (sum of temperature degrees), provide a very clear picture of their earliness, very important information in the process of hybridization and obtaining hybrids in the current climatic context.

The total plant height is in a medium direct correlation with the height of the main cob insertion, with the length of the main cob leaf and a weak direct correlation with the width of the main cob leaf.

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WINTER WHEAT VARIABILITY UNDER ETHYLMETHANSULFONATE ACTION

Vladislav HORSHCHAR, Mykola NAZARENKO

Dnipro State Agrarian and Economic University, 25 S. Efremova Street, Dnipro, Ukraine

Corresponding author email: nik_nazarenko@ukr.net

Abstract

The object of research was the hereditary variability of a group of genotypes, selected in order to maximally characterize the existing biological diversity of winter wheat cultivated in Ukraine. Winter wheat dry seeds of eight varieties were acted with water (control) and EMS (ethylmethansulfonate) action in concentrations of 0.025%, 0.05%, 0.1%. An extremely high general mutation rates and variability in the spectra of changes was observed. The level of variability showed that the concentration of 0.05% was more promising in terms of the number of altered traits; it was also successful in inducing predominantly agronomical-valuable traits. (earliness, forms with long well-grained spike, short stem, semidwarf, with high photosynthetic ability, lines with high grain productive. New promising lines were identified with improved plant architecture, optimal ratio between grain and vegetative part of the plant (index of economic value). A further increase in the concentration of the mutagen to 0.1% only led to a decrease in variability and a smaller number of valuable forms. However, it can be used to obtain dwarf forms and forms with systemic changes in the spike.

***Key words**: winter wheat, chemical mutagenesis, ethylmethansulfonate, mutation, plant improvement.*

INTRODUCTION

Winter wheat is a key food crop for Ukraine and the world (Andrusevich et al., 2018). Taking into account the problems of climate change, gradual migration and advancement of crops to the south in areas that previously did not guarantee stable high yields, the genetic improvement of this crop acquires a new strategic importance (Beiko and Nazarenko, 2022).

One of the options for stable improvement is induced biodiversity through the use of appropriate mutagenic factors (Yakymchuk et al., 2021; Shabani et al., 2022).

The utilization of factors that induce genetic diversity is associated with the effect of the so-called mutagenic depression (Nazarenko et al., 2022), which leads to significant problems with increasing the dose or concentration of this mutant agent, given the need to obtain a sufficient amount of viable fertile material for research (OlaOlorun et al., 2020).

One of the main ways of solving this problem are the use of less harmful agents (so-called supermutagens), which, with an increase in mutational activity in 20-60 times (Mangi et al., 2021), do not exceed more traditional factors such as gamma-rays or fast neutrons in terms of the level of physiological or genetical damages (Spencer-Lopes et al., 2018).

The use of site-specific mutagenesis with the establishment of the mechanism of specific factor nature action makes it possible to obtain new changes in forms with stable properties and traits useful from a breeding or genetic point of view (Nazarenko et al., 2020), in particular, valuable changes in the biochemical structure, in a short period (Nazarenko and Lykholat, 2018).

Chemical factors show significant site-specificity, relationship to certain areas of the hereditary substance, which leads to the predominant induction of only certain types of traits (Yali and Mitiku, 2022).

The investigation of the variety-specific activity of mutagens is carried out from the first generation, since the influence on the indicators of growth and development (Nazarenko et al., 2020), the formation of the plant organism occurs precisely at this stage (Ram et al., 2019).

The use of chemical mutagenesis to induce genetic diversity leads mainly to small changes in key traits, unlike physical mutagens, the changes are complex, and the lethal effects are significantly low (Shabani et al., 2022).

More useful in modern research is the possibility to get large samples of material with low damages without the use of substances with high continuous activity (Beiko and Nazarenko, 2022), but for some agents the tendency may be the opposite, especially in the studies of mechanisms of some value traits genetic control (Anter, 2021).

The key is the process of interaction between the genotype of the variety and nature/concentration of mutagen, taking into account the threshold action of agent concentrations with lower damaging activity and site-specific capacity. Not only a decrease in the number of valuable changes may not occur, but they can be more likely if the optimal combinations of concentration, the nature of mutagen and the genotype of the initial form (le Roux et al., 2021).

The main purpose of our experiments was to characterize the genetic and phenotypic variability of winter wheat families after mutagen action with identification stable lines by key traits, evaluation of role genotype and mutagen interactions for new complex changes and new type of mutations. The first target was relations between initial material and nature of chemical substance, its concentrations. Second our aim was to identify mutagen effect and mutant lines suitability for future winter wheat improvement.

MATERIALS AND METHODS

The experiment has been conducted under the conditions of the experimental fields station of the Science-Education Center of the Dnipro State Agrarian Economic University during 2017-2022.

Winter wheat seeds (1000 grains for each concentration and water) were acted with a EMS (ethylmethansulfonate) 0.025%, 0.05%, 0.1% (Sigma-Aldrich, Germany). Seeds has been treated with an exposition of 24 hours in order to with the generally recommended method for chemical mutagens actions protocol. These concentrations are trivial for mutagens (chemical supermutagens) of this group. The control was soaked in water (Shu et al., 2013; Spencer-Lopes et al., 2018).

Seeds samples were sown for 32 variants (in total) (10-rows plots for every variant, in water as control, interrow-spacing was 0.15 m, length of row was 1.5 m) by varieties (ecotypes in brackets FS - forest-steppe, all for all zones, S - steppe) Balaton (FS), Borovytsia (all), Zeleny Gai (S), Zoloto Ukrainy (FS), Kalancha (all), Niva Odeska (all), Polyanka (all), Pochayna (all). The genotypes were identified according to characterize winter wheat varieties variability for North Steppe subzone (Dnipro region) (Spencer-Lopes et al., 2018).

The agrotechnology of crop cultivation is trivial for the Steppe zone (semi-arid area).

Sowing material were grown in rows with inter and intra-row spacing of 50 and 30 cm, respectively, for first generation of families. The control was sample of non-treated grains of parent varieties and one were also grown after ten rows for each variant as comparison with the first-generation plants and next generations families. Mutant lines and groups rows were planted at three replications with control-rows of parent variety for each twenty-row plot (Mangi et al., 2021).

At M_2-M_3 generations mutation families have been selected via visual evaluation. The sowing was done by hand, at the end of September, at a depth of 4-5 cm and with a rate of 100 viable seeds to a row (length 1.5 m), interrow was 15 cm, between samples 30 cm, 2 rows for sample with control-row of initial variety samples after every 20 variants.

Field experiment was conducted at the Science-Education Center of Dnipro State Agrarian and Economic University (48°51′10″ n. l. 35°25′31″ e. l.). Trivial for zone agricultural practices including fertilization were provided. Estimation was conducted during 2017-2022 years.

Statistic analyze of data was performed by ANOVA-analysis, grouping and estimation of data was provided by discriminant and cluster analysis (Euclidian distance, single linkage) (Statistic 10.0, multivariant module, TIBCO, Palo Alto, USA). The normality of the data distribution was examined using the Shapiro–Wilk W-test. Differences between samples were assessed by Tukey HSD test.

RESULTS AND DISCUSSIONS

In total, for control and material after mutagen action 15800 families at second-third generation were investigated. The concentrations of mutagen characteristic for breeding practice have been used. The number of families on average was about 500 per variant. The exception is the extreme concentration (EMS 0.1%), for some more susceptible varieties as Balaton, Niva Odeska, the sample can be 400 families.

Data about the general mutation rate for each variety and variant are presented at Tables 1 and 2 in such a way that the more susceptible varieties (subsequently it will be shown by grouping by clusters) were at Table 1, and significantly less variable at Table 2.

Table 1. General rate of mutations cases and families at second - third generations. First group (more sensitive to mutagen action) (x ± SD, n = 400-500)

Variety	Number of selecting families	Number of mutant families	Rate of mutations, %
Balaton	500	2	0.40 ± 0.10[a]
Balaton, EMS 0.025%	500	34	6.80 ± 0.32[b]
Balaton, EMS 0.05%	500	45	9.00± 0.45[c]
Balaton, EMS 0.1%	400	60	15.00±0.60[d]
Zoloto Ukrainy	500	6	1.20 ± 0.24[a]
Zoloto Ukrainy, EMS 0.025%	500	38	7.60± 0.52[b]
Zoloto Ukrainy, EMS 0.05%	500	55	11.00±0.80[c]
Zoloto Ukrainy, EMS 0.1%	500	71	14.20±0.90[d]
Kalancha	500	5	1.00 ± 0.20[a]
Kalancha, EMS 0.025%	500	31	6.20±0.30[b]
Kalancha, EMS 0.05%	500	41	8.20±0.35[c]
Kalancha, EMS 0.1%	500	65	13.00±0.50[d]
Niva Odeska	500	3	0.60 ± 0.18[a]
Niva Odeska, EMS 0.025%	500	35	7.00±0.35[b]
Niva Odeska, EMS 0.05%	500	48	9.60±0.48[c]
Niva Odeska, EMS 0.1%	400	64	16.00±0.75[d]

Note: indicate significant differences at P < 0.05 by ANOVA-analyse with Bonferroni amendment. Comparison in terms of one variety.

As can be seen (in addition), the more variable varieties were also among those that showed higher depressive effects on growth and development in the first generation. There were varieties Balaton (general rate up to 15%), Zoloto Ukrainy (up to 14.2%), Kalancha (up to 13%), Niva Odeska (up to 16%).

As a result of factor analysis, a statistically significant difference from the varieties of the second group was established ($F = 12.17$; $F_{0.05} = 5.16$; $P = 0.01$), frequencies at the highest concentration vary from 13 to 16%, while in Table 2 the general rate of mutations at the same concentration was for the genotypes of the second group for the varieties Borovytsia (10.2%), Zeleny Gai (9.6%), Polyanka (8.8%) Pochayna (9.2%), that is at the level of 8.8-10.2%, which, firstly, is significantly lower than that for the varieties of the first group, and secondly, the variability within the group is also much lower.

Table 2. General rate of mutations cases and families at second - third generations. First group (more tolerance by genetic activity) (x ± SD, n = 400-500)

Variety	Number of selecting families	Number of mutant families	Rate of mutations, %
Borovytsia	500	4	0.80 ± 0.08[a]
Borovytsia, EMS 0.025%	500	28	5.60±0.30[b]
Borovytsia, EMS 0.05%	500	37	7.40±0.45[c]
Borovytsia, EMS 0.1%	500	51	10.20±0.68[d]
Zeleny Gai	500	3	0.60 ± 0.06[a]
Zeleny Gai, EMS 0.025%	500	27	5.40±0.30[b]
Zeleny Gai, EMS 0.05%	500	36	7.20±0.45[c]
Zeleny Gai, EMS 0.1%	500	48	9.60±0.50[d]
Polyanka	500	2	0.40 ± 0.12[a]
Polyanka, EMS 0.025%	500	23	4.60±0.40[b]
Polyanka, EMS 0.05%	500	33	6.60±0.50[c]
Polyanka, EMS 0.1%	500	44	8.80±0.55[d]
Pochayna	500	2	0.40 ± 0.14[a]
Pochayna, EMS 0.025%	500	24	4.80±0.35[b]
Pochayna, EMS 0.05%	500	32	6.40±0.40[c]
Pochayna, EMS 0.1%	500	46	9.20±0.55[d]

Note: indicate significant differences at P < 0.05 by ANOVA-analyse with Bonferroni amendment. Comparison in terms of one variety.

However, for both groups mutagen action was statistically significant both for the variance in the change in mutagen concentration ($F = 127.23$; $F_{0.05} = 3.86$; $P = 2.17*10^{-9}$) and for individual genotypes ($F = 55.16$; $F_{0.05} = 3.86$; $P = 1.19*10^{-5}$).

At all cases, the differences are statistically significant for all concentrations in all varieties, regardless of the group, both in relation to the control and in relation to the effect of the previous concentration (Tables 1 and 2, respectively).

In general, all varieties belong to stable genotypes and the level of spontaneous variability is low, moreover, as for modern varieties, for which a significantly lower genome stability is noted, it is even low.

The cluster analysis carried out by the total mutation frequency showed (Figure 1) that, in general, varieties are quite clearly divided into two groups according to the genotype-mutagenic interaction.

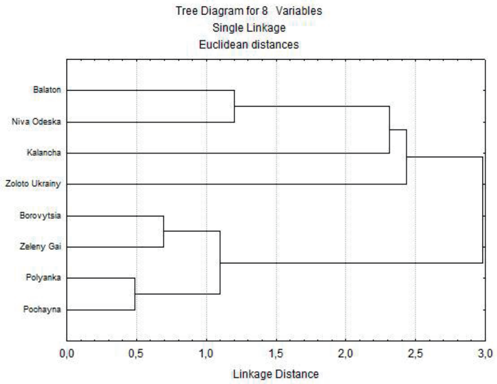

Figure 1. Results of cluster analysis by general mutation rate

Thus, at first group are more susceptible to EMS action varieties Balaton, Kalancha, Niva Odeska; at second group varieties Borovytsia, Zeleny Gai, Polyanka, Pochayna. Variety Zoloto Ukrainy stood out a little apart, which, although it belonged to the first group in the analysis of the date from tables, stood out in a special minor group after the cluster analysis. Apparently, this is due to the dynamics of the change in general rate depending on the concentration of the mutagen; there can be no other reasons.

More interesting was the investigation by the integrative parameter of the level of variability, which takes into account not only the general rate of variability under the mutagenic factor action, but also the width of the spectrum of changes, i.e., the number of traits that undergo changes under the action of a given concentration (Table 3 for the first group of varieties and Table 4 for the second group, the cluster analysis data for this parameter are presented in Figure 2).

Based on the presented data, we find that there are also statistically significant changes with each concentration ($F = 179.13$; $F_{0.05} = 3.86$; $P = 3.02*10^{-12}$) and depending on the genotype of the initial material ($F = 73.18$; $F_{0.05} = 3.86$; $P = 2.07*10^{-7}$), we also again find differences between the two groups of varieties ($F = 18.34$; $F_{0.05} = 5.16$; $P = 0.002$).

At the same time, for the first group, the level of variability was at the highest concentration from 3.77 (сорт Kalancha) to 5.12 (сорт Niva Odeska), for second (more resistance group for action) group from 2.11 (variety Polyanka) to 2.69 (variety Zeleny Gai).

Table 3. Level of changeability, caused by mutation variability. First group (x ± SD, n = 400-500)

Variant	Level of variability	Changed traits
Balaton	0.01±0.01[a]	2
Balaton, EMS 0.025%	1.43±0.11[b]	21
Balaton, EMS 0.05%	2.34±0.23c	26
Balaton, EMS 0.1%	4.50±0.25[d]	30
Zoloto Ukrainy	0.07±0.02[a]	6
Zoloto Ukrainy, EMS 0.025%	1.37±0.14[b]	18
Zoloto Ukrainy, EMS 0.05%	2.86±0.22[c]	26
Zoloto Ukrainy, EMS 0,.1%	4.12±0.29[d]	29
Kalancha	0.05±0.01[a]	5
Kalancha, EMS 0.025%	1.12±0.09[b]	18
Kalancha, EMS 0.05%	1.89±0.21[c]	23
Kalancha, EMS 0.1%	3.77±0.27[d]	29
Niva Odeska	0.02±0.01[a]	3
Niva Odeska, EMS 0.025%	1.61±0.11[b]	23
Niva Odeska, EMS 0.05%	2.69±0.23[c]	28
Niva Odeska, EMS 0.1%	5.12±0.31[d]	32

Note: indicate significant differences at $P < 0.05$ by ANOVA-analyse with Bonferroni amendment. Comparison in terms of one variety.

Table 4. Level of changeability, caused by mutation variability. Second group (x ± SD, n = 400-500)

Variant	Level of variability	Changed traits
Borovytsia	0.03±0.01[a]	4
Borovytsia, EMS 0.025%	0.95±0.08[b]	17
Borovytsia, EMS 0.05%	1.63±0.17[c]	22
Borovytsia, EMS 0.1%	2.65±0.25[d]	26
Zeleny Gai	0.02±0.01[a]	3
Zeleny Gai, EMS 0.025%	1.03±0.11[b]	19
Zeleny Gai, EMS 0.05%	1.66±0.18[c]	23
Zeleny Gai, EMS 0.1%	2.69±0.22[d]	28
Polyanka	0.01±0.01[a]	2
Polyanka, EMS 0.025%	0.64±0.07[b]	18
Polyanka, EMS 0.05%	1.25±0.17[c]	23
Polyanka, EMS 0.1%	2.11±0.21[d]	29
Pochayna	0.01±0.01[a]	2
Pochayna, EMS 0.025%	0.91±0.09[b]	14
Pochayna, EMS 0.05%	1.41±0.12[c]	19
Pochayna, EMS 0.1%	2.39±0.19[d]	24

Note: indicate significant differences at $P < 0.05$ by ANOVA-analyse with Bonferroni amendment. Comparison in terms of one variety.

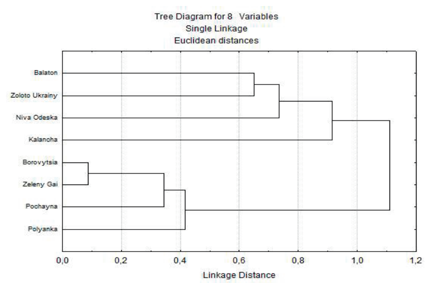

Figure 2. Results of cluster analysis by level of variability

Again, it finds that the variability within the second group is significantly lower, but the differences between the groups are very significant even at the initial assessment. Cluster analysis in this case showed a clearer division into two groups (Figure 1) without the presence of any minor options, while the factor space of the analysis is significantly smaller than in the first case. Thus, it can be considered mathematically justified that the estimate is more accurate in terms of the level of variability than in terms of the total rate of mutations.

In the spectrum of changes obtained, the traits were divided into 6 groups in accordance with the generally accepted classification. A classification analysis was also carried out both for individual characteristics of the mutation process (Tables 5 and 6) and for genotypes (varieties) (Table 7).

Table 5. Results of discriminant analyze

Variables at model	Wilks Lambda λ	Partial Lambda	F-remove (4,02)	p-level
Mutation rate	0.11	0.79	17.23	0.01
Level of variability	0.07	0.92	21.14	0.01
First group	0.14	0.70	8.92	0.01
Second group	0,57	0,31	1,99	0.13
Third group	0,32	0,55	3,69	0,06
Fourth group	0.11	0.78	16.17	0.01
Fifth group	0.19	0.61	5.14	0.03
Sixth group	0,39	0,47	2,93	0,09

The first group of mutations in plant architecture. These include the following signs of thick stem, thin stem, high stem, short stalk, semidwarf, dwarf, intensive waxy bloom, weak waxy bloom and the presence of a waxy bloom. On the whole, in the group, the most highly probable for EMS action are such forms as high stem, semidwarfs (at high concentrations) and forms with a weak waxy bloom. As can be seen, less pronounced mutations are more characteristic, with the exception of semi-dwarfs (but the latter is due to the fact that the initial material was predominantly short). The highest (up to 1.5%) probability of the appearance of high forms, which are present in almost every variant.

The second group includes changes in wheat grain. Such traits as barrel-shaped grain, coarse grain, fine grain was revealed. Only the large grain mutation occurs more or less often (and its probability increases slightly with increasing concentration in some varieties), other mutations are rare. The group is difficult to identify and has little weight in the factor space.

The third group includes changes in the spike structure (the most numerous, 15 different traits). These changes tend to occur more frequently as the concentration increases. Some varieties are characterized by the presence of a greater number of such changes as anthocyanin awns and a double spike. The mutation of the spike from awn spike to awnless form (almost three times) is more frequent than from awnless spike to with awn. Transitional forms (semi-awn spike) occur at the level of the second variant.

The fourth group (changes in the physiology of growth and development) is the most variable. Only 4 traits as sterility, early-maturing, late-maturing, disease tolerance. More frequent (for all variants) are changes in early maturity and disease tolerance. At the same time, for some variants, especially with an increase in concentration, late maturing may become. Sterility is more typical for high concentrations and practically does not occur at low concentrations. In general, all traits for this group were in model.

The fifth group includes systemic mutations that lead to extremely significant changes in the spike structure, going beyond the cultural form and leading to the phenotype of wild relatives. Such changes are most probable under the action of high concentrations of the mutagen and practically do not occur at low ones. More likely is the appearance of squarehead, which can form even at low concentrations. Other mutations are quite rare.

The sixth group consists of valuable forms by grain productivity and tillering. It occurs in most varieties, except for some varieties at a concentration of EMS 0.1%.

Modeling of individual parameters by groups was established for the mutation process in the course of discriminant analysis (Table 5). The model was the frequency, the level of variability, mutations in the first, fourth, fifth group.

Thus, it is possible to reliably predict for a given mutagen on a given material how to obtain sources for breeding for early maturity, disease resistance, plant height, but it is difficult to find productive forms. As for the significance for the implementation of one or another trait depending on the genotype and concentration of the mutagen (Table 6), the value of the mutagen concentration is still of great importance, but only in the case of the last group there was no influence of the genotype.

Table 6. Factor loadings (varimax raw)

Parameter	Genotype	Concentration
Mutation rate	**0.616148**	**0.923626**
Level of variability	**0.783614**	**0.875313**
First group	**0.622911**	**0.776561**
Second group	0.357780	0.322229
Third group	**0.766511**	**0.728113**
Fourth group	**0.674513**	**0.796612**
Fifth group	**0.550213**	**0.901179**
Sixth group	0.321734	**-0.448767**
Expl.Var	2.935617	3.347610
Prp.Totl	0.453231	0.379119

Note: statistically significance in bold

Significantly, both the genotype of the initial material and the concentration of the mutagen affected on the mutation rate, the level of variability and the frequencies for the first and third-fifth groups of mutations, which somewhat contradicts the results of discriminant analysis, supplementing it with new significant parameters (the third group for mutations).

The classification by varieties in factor space showed that all genotypes are reliably identified by the obtained parameters, Zoloto Ukrainy variety is least of all. In general, varieties more susceptible to the mutagen are better classified (Table 7).

Table 7. Classification matrics - canonical roots

Genotype	Percent of classification
Balaton	100.0
Zoloto Ukrainy	75.0
Kalancha	100.0
Niva Odeska	100.0
Borovytsia	87.5
Zeleny Gai	100.0
Polyanka	87.5
Pochayna	87.5
Total	92.2

The obtained results make it possible to evaluate the possibilities of chemical mutagenesis and the use of highly active substances in terms of the induction of genetic changes to obtain new forms (Mangi et al., 2021; Hassine et al., 2022) or making adjustments to the initial material (variety or line), which generally meets to modern requirements, but needs to be improved for some parameters (Abaza et al., 2020).

This mutagen is quite actively used in practice to obtain new genetically- and breeding-value forms. (OlaOlorun et al., 2021), primarily due to the high variability in terms of changes in plant structure (plant architecture) (OlaOlorun et al., 2020; le Roux et al., 2021), the formation of plants that better meet to modern requirements for intensive varieties (Ram et al., 2019) through the transformation of local semi-intensive, but better adapted forms (under our conditions, local varieties of national breeding) (le Roux et al., 2021).

On the other hand, the investigated mutagen on this material showed a slightly different range of changes than in the case of foreign scientific programs (OlaOlorun et al., 2021). If the general mutation rate is quite significantly higher, primarily for the group of genotypes identified as less resistant to a factor of this nature, then the proportion of beneficial changes is less significant than expected (Hassine et al., 2022; Nazarenko et al., 2022). Perhaps, more thorough investigations of grain quality and various types of tolerance to unfavorable environmental factors for mutant lines of older generations will significantly correct the results (Chaudhary et al., 2019; Hong et al., 2022).

It should also be noted that there is a rather strong relationship between the frequency of chromosomal aberrations obtained for this material and the frequency of visually determined mutations. Although this effect has been noted in the past, but not always (Yakymchuk et al., 2021; Yali and Mitiku, 2022).

The use of integrative indicators, which, in addition to characterizing the total number of mutant cases, also includes the width of the spectrum of change by the number of individual traits affected by the genetic activity of the factor, was previously noted by us as more promising (Ram et al., 2019; Shabani et al., 2022; Nazarenko et al., 2022). This was confirmed primarily for this mutagen, even with greater justification through mathematical and statistical analysis than previously for gamma-rays (Nazarenko, 2017), nitrosoalkylureas, or other mutagenic factors (Nazarenko et al., 2022).

CONCLUSIONS

EMS as a mutagenic factor shows an extremely significant dependence on the characteristics of the genotype-mutagenic interaction, i.e. depends on the characteristics of the initial material genotype with a clear subdivision according to genetically determined reactivity to the action of this mutagenic factor.

When observing all types of effects of mutagenic action from the cellular to the whole plant level, there is a clear relationship in terms of the severity of the impact of this factor, which is not always the case. This indicates the nature of a rather direct (through a direct effect on the DNA structure), rather than an indirect effect on the hereditary apparatus of a plant cell.

The most important is a comprehensive assessment of the general rate and spectrum of the obtained changes, which shows the success of this mutagen at the induction of mutations (Spencer-Lopes et al., 2018). Consideration of only one component is meaningless and may lead to the loss of part of the data for the classification analysis (OlaOlorun et al., 2020).

Investigated mutagen is more valuable in terms of inducing a wide range of different changes than in terms of obtaining economically valuable forms and lines, at least at the level of visual identification of mutations (Yakymchuk et al., 2021). This allows us to consider it more promising for clarifying data on the control mechanisms of certain traits and obtaining a number of forms that can be used in the future not directly as commercial varieties, but as a source of some valuable key traits for the partial improvement of existing varieties and lines.

It is planned to conduct a study of the selected promising material on tolerance to adverse environmental factors (winter period, drought resistance, hot resistance), assess the technological qualities of grain (protein content, low- and high-molecular glutenins, gliadins), show possible changes in the content of valuable microelements in wheat grains.

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MYCOTOXIN CONTENT IN MAIZE IN THE CASE OF *Fusarium graminearum* INOCULATION IS REDUCED AFTER FUNGICIDAL TREATMENT

Atanas IVANOV, Adelina HARIZANOVA

Agricultural University of Plovdiv, 12 Mendeleev Blvd, Plovdiv, Bulgaria

Corresponding author email: ativanov@icloud.com

Abstract

The present study aims to investigate the effect of two pesticide products against Gibberella ear rot (Fusarium graminearum) on yield quality and quantity of maize (Zea mays L.) and choose the more appropriate application dose and time. Three field experiments were performed in the period 2019-2021 in the Plovdiv region, Bulgaria. Three different varieties of maize were used. The pesticides used were Propulse 250 SE (prothioconazole 125 g/l + fluopyram 125 g/l) - applied in two doses - 0.6 l/ha or 1 l/ha and Prosaro 420 SC (prothioconazole 210 g/l + tebuconazole 210 g/l) - applied in only one dose - 1 l/ha. Seven variants, including untreated control and six different treatments, were analyzed. The treatments were divided into two groups. To understand which application moment was more appropriate, half of the plots were treated before the inoculation, and the others - during the sporulation phase of the pathogen. The results showed a significant reduction in the disease severity and incidence after the application of both products. There were also differences in yield quantity and thousand kernel weight, hectoliter mass, and the content of deoxynivalenol.

Key words: *deoxynivalenol, gibberella ear rot, maize, prothioconazole, yield.*

INTRODUCTION

Human health and nutrition, as well as animal feed, are major priorities of farmers all over the world. Providing high-quality production could be a challenge, especially when the pesticide application should be reduced year after year.
One of the most important plant pathogens that attacks a wide range of plant species, including maize (*Zea mays* L.) (ear and stalk rot), wheat and barley (head blight), is *Fusarium graminearum* (sexual stage *Gibberella zeae*) (Harris et al., 1999). The infection leads to a reduction of both yield and grain quality. The reduction in quality is partially a result of the mycotoxins produced by this fungus. Usually, the ears which were infected with *Gibberella* ear rot contain the trichothecenes deoxynivalenol (DON), 15-acetyldeoxynivalenol (15AcDON), and 3-acetyldeoxynivalenol (3AcDON), as well as zearalenone (Mirocha & Christensen, 1974; Miller et al., 1983; Bennett et al., 1988) and fusarin C (Farber & Sanders, 1986).
Mycotoxins are secondary metabolites synthesized by molds. These substances adversely affect humans, animals, and crops, resulting in illnesses and economic losses (Zain, 2011). Aflatoxins, ochratoxins, trichothecenes, zearalenone, fumonisins, tremorgenic toxins, and ergot alkaloids are the mycotoxins of greatest agro-economic importance. Some molds could produce more than one mycotoxin, and many of the mycotoxins are produced by more than one fungal species.
The contamination of human food can happen at different stages in the food chain (Bennett and Klich, 2003).
The main toxic effect of trichothecene mycotoxins (TCT) is the primary inhibition of protein synthesis. These substances commonly contaminate poultry feed and feedstuffs (Leeson et al., 1995), where they could affect cells, which divide actively like the cells in the gastrointestinal tract, the skin, lymphoid and erythroid cells. After contact with the mycotoxin a necrosis of the oral mucosa and skin appears. An adverse effect on the digestive tract and decreased bone marrow and immune function is also observed (Schwarzer, 2009).
Using fungicides on grain corn or silage aims to control fungal diseases such as *Gibberella* ear rot (*Fusarium graminearum*). Several

studies have demonstrated that applying fungicides at the proper stage reduces DON contamination in corn (Edwards et al., 2001; Cardoso, 2020). On the contrary, others suggest that the use of some chemicals could stimulate DON synthesis as a defense mechanism against pesticide-induced stress (Magan et al., 2002).
The maximum limit (ML) for DON in maize grain for further processing by procedures proven to reduce DON levels was agreed upon at 2,000 μg/kg (Schaarschmidt & Fauhl-Hassek, 2021).
Different agricultural practices could be applied to reduce the adverse effect of pathogen infection (Edwards, 2004). Some are focused on developing resistant varieties (Kolb et al., 2001; Rudd et al., 2001; Snijders, 2004). Others recommend crop rotation (Dill-Macky & Jones, 2000). According to many researchers, using pesticides with a fungicidal effect could, to some extent, alleviate the negative effects of the pathogen (Wise & Mueller, 2011; Luna & Wise, 2015).
Several active ingredients were examined in the last years. Prothioconazole is a demethylation-inhibiting fungicide (DMI) that blocks or prevents the fungus from producing essential sterol compounds, such as ergosterol, that are important for fungal membrane and structure (Latin, 2011; Mueller et al., 2013). Tebuconazole is also a DMI fungicide and acts the same way as prothioconazole.
Fluopyram is a pyridyl ethyl benzamide broad-spectrum fungicide and nematicide. It belongs to the group of Succinate dehydrogenase inhibitors (SDHIs) – a class of fungicides that act on succinate dehydrogenase and inhibit the respiration of pathogenic fungi (Xu et al., 2019). Fluopyram is effective against parasitic nematodes and soil-borne fungi (Nadeethara Lohithaswan et al., 2022).

MATERIALS AND METHODS

Experimental design

Three field experiments were performed in the period 2019-2021. The trials were set up in the Region of Plovdiv using the block plot method with a plot size of 30 m^2. Seven variants including untreated control, Propulse 250 SE in two dose rates (0.6 l/ha and 1 l/ha), and Prosaro (1 l/ha) were tested. Propulse 250 SE (Bayer Crop Science) contains prothioconazole 125 g/l and fluopyram 125 g/l. Prosaro 420 SC (Bayer Crop Science) contains prothioconazole 210 g/l and tebuconazole 210 g/l. The details about the applications are presented in Table 1.

Table 1. Application data

Variants	Application	Product
1 (Control)	-	-
2	Application A (foliar application before inoculation)	Propulse 250 SE 0.6 l/ha
3	Application A (foliar application before inoculation)	Propulse 250 SE 1 l/ha
4	Application A (foliar application before inoculation)	Prosaro 420 SC – 1l/ha
5	Application B (foliar application during pathogen sporulation)	Propulse 250 SE 0.6 l/ha
6	Application B (foliar application during pathogen sporulation)	Propulse 250 SE 1 l/ha
7	Application B (foliar application during pathogen sporulation)	Prosaro 420 SC – 1 l/ha

Experimental design 2019

In 2019 the trials were set up in Purvenets (GPS 42.095237 and 24.677521). The experiment was performed with maize cv. DKS5075 after oats (*Avena sativa* L.) as a previous crop. The seeds were sown on 28 March 2019, with a sowing rate of 68,000 plants/ha with a row spacing of 70 cm and space within row of 18.6 cm. Every plot included four rows. The plants emerged on 9 April 2019. The first application (Application A) with the test products was performed on 19 June 2019. The air temperature during the application was 18.3°C, the relative humidity - 59%, the wind velocity - 0.3 m/s, the cloud cover was 0%, and the soil temperature was 24.7°C. The developmental stage of the plans during the application was BBCH 61 (Majority), and the plant height at that time was 215 cm. The second application (Application B) was performed 11 days after the first one, on 1 July, during the sporulation phase of the pathogen. The air temperature was 23.2°C, the relative humidity - 70%, the cloud cover - 10%, the wind velocity - 0.3 m/s, and the soil temperature was 26.6°C. The plants were treated in BBCH stage 67 (Majority) and the plant height at that time was 220 cm.

Experimental design 2020

In 2020, the experiment was performed in the village of Katunica (GPS 42.077679 and 24.676725) with maize cv. SY Phenomen after sunflower (*Helianthus annuus* L.) as a predecessor. The seeds were sown on 3 April and the young plants emerged on 18 April. The row spacing was 70 cm, and the space within row was 18.1 cm (four rows per plot). The sowing rate was 70,000 plants/ha. The first application of the test products (Application A) was performed on 25 June 2020 before the inoculation. The air temperature during the application was 19.1°C, the relative humidity - 57%, the cloud cover - 20%, the wind velocity - 1.7 m/s, and the soil temperature was 25°C. The developmental stage of the plants was BBCH 61 (Majority), and the plant height was 173 cm. The second application (Application B) was performed 12 days after the first one, on 7 July 2020, during the sporulation phase. The air temperature during the application was 22.1°C, the relative humidity - 59%, the cloud cover - 10%, the wind velocity - 0.3 m/s, and the soil temperature was 26.4°C. The developmental stage of the plants during the application was BBCH 69 (Majority), and the plant height was 215 cm.

Experimental design 2021

In 2021, the experiment was performed in the village of Trud (GPS 42.191797 and 24.722284) with maize cv. ES Faradi. The seeds were sown on 13 April after winter barley (*Hordeum vulgare* L.) as a predecessor, and the young plants emerged on 4 May 2021. The row spacing was 70 cm, and the space within row was 18.1 cm. Every plot had four rows. The sowing rate was 80,000 plants/ha. The first application with the test products (Application A) was performed on 13 July 2020. The air temperature during the application was 23.1°C, the relative humidity was 63%, the cloud cover was 50%, the wind velocity - 1.7 m/s, and the soil temperature was 25.5°C. The developmental stage of the plants during the application was BBCH 63 (Majority), and the height of the plants was 200 cm. The second application was performed six days after the first one, on 19 July 2021, during the sporulation phase. The air temperature during the application was 21.9°C, the relative humidity was 53%, the cloud cover - 20%, the wind velocity - 1.1 m/s, and the soil temperature was 25.3°C. The developmental stage of the plants during the application was BBCH 67 (Majority) and the plant height was 220 cm.

Fusarium graminearum inoculation

The artificial inoculation with *Fusarium graminearum* was performed at BBCH Stage 65 with a spray concentration of 10^3 conidia/ml. The leaves, tassels, and the stems of the plants on every experimental plot were uniformly sprayed with a volume spray of 800 l/ha three days after the first fungicide treatment.

Mycotoxin estimation

The content of deoxynivalenol (DON) was measured using high-performance liquid chromatography (HPLC) with tandem mass spectrometry (MS/MS) meeting VLM 92 2010.

Statistical data analysis

The data are presented as the mean of four replicates. The experimental results were statistically processed with the SPSS program using a one-way ANOVA dispersion analysis and Duncan's comparative method, with the validity of the differences determined at a 95% significance level. The different letters (a, b, c, d) after the mean value show statistically significant differences between the variants.

RESULTS AND DISCUSSIONS

During the first experimental year, the disease severity (% of the infected area on corn cobs) and disease incidence (% of the plants with disease symptoms) were evaluated on 6 August 2019. The analysis of the disease severity and disease incidence in 2019 is presented in Figure 1. The results show that the disease severity and incidence were the highest in the untreated plots. The disease severity in variant 2 (Propulse 250 SE 0.6 l/ha, applied before the inoculation) was 27% lower than the control. In variant 5, where Propulse 250 SE was applied at the same dose of 0.6 l/ha, but in the sporulation phase, the disease severity was reduced by 34% compared to the control. The severity was the lowest in variant 3 (Propulse 250 SE 1 l/ha before inoculation), variant 4 (Prosaro 420 SC before inoculation), variant 6 (Propulse 250 SE 0.6 l/ha during sporulation phase), and variant 7 (Prosaro 420 SC during sporulation phase). The reduction was by 62%, 63%, 59%, and 65%, espectively. The effect of pesticides on disease incidence was similar. The disease incidence was reduced by 27% in variant 2 and by 25% in variant 5, respectively. The reduction of the disease incidence in variants 3, 4, 6, and 7 was by 56%, 69%, 63%, and 66%, respectively.

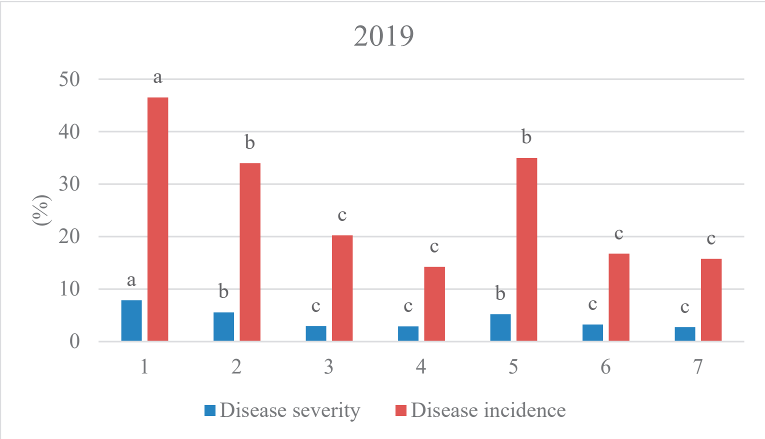

Figure 1. Disease severity and disease incidence of *Fusarium graminearum* in maize, cv. DKS5075 in 2019

The results show that in 2019 the effect of the applied products depended on the application dose and was not related to the moment of application. The higher application dose resulted in a better ability to control the disease severity and incidence. The lower dose of the test product Propulse 250 SE (0.6 l/ha) reduced the disease severity and disease incidence compared to the untreated plots, but the higher dose of application (1 l/ha) of both products led to a bigger reduction of the disease severity and incidence.

Regarding disease incidence, the tendency was different. The untreated plants were still the ones that were most affected by the pathogen. In this case, the rate of damage on the pesticide-treated plots was affected not only by the application dose but also by the application time. It was observed that the disease incidence was lower on the test plots, which were treated before inoculation (Application A), compared to the variants which were treated during the sporulation phase (Application B). The preventive treatment resulted in a better protective effect of the tested products. The pretreatment with Propulse 250 SE 0.6 l/ha (variant 2) led to a decrease in the disease incidence by 56%, and the treatment in the sporulation phase with the same application dose (variant 5) resulted in a 33% reduction. The application of Propulse 250 SE in the dose of 1 l/ha (variant 3) reduced the disease incidence by 89% vs. 75% when the same dose was applied during the sporulation phase. The treatment with Prosaro 420 SC reduced the disease incidence by 77% when applied before the inoculation and by 54%, respectively, when the plots were treated during the sporulation phase. The same tendency was observed also in the third experimental year. The disease severity and incidence during 2021 were measured on 18 August 2021. The data are presented in Figure 3. The results show that the highest disease severity and incidence were measured on the untreated test plots. The disease severity on plots that were treated with Propulse 250 SE 0.6 l/ha before the inoculation was 62% lower than the control. The rate of 1 l/ha Propulse 250 SE led to a 94% decrease. The application of Prosaro 420 SC decreased the severity by 90%. When Propulse 250 SE 0.6 l/ha was applied during sporulation, the reduction was by 63%, and by 93%, when the higher dose of 1 l/ha was applied, respectively. The treatment with Prosaro 420 SC during sporulation led to a decrease in the disease incidence by 83% compared to the untreated control.

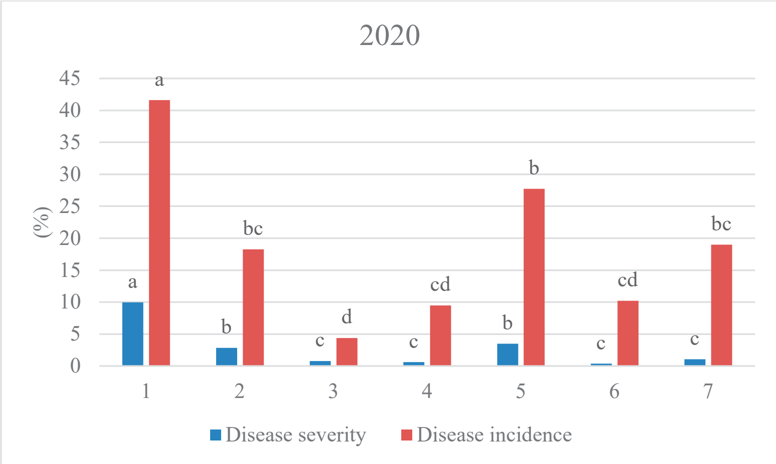

Figure 2. Disease severity and disease incidence of *Fusarium graminearum* in maize, cv. SY Phenomen in 2020

In 2020 the disease severity and disease incidence were measured on 11 August 2020. The results are presented in Figure 2. It is seen that the disease severity and incidence were the highest in the untreated control. The disease severity was reduced by 71% in variant 2 and by 65% in variant 5 respectively, compared to the control. The damage was the lowest in variants 3, 4, 6, and 7. The reduction was by 92%, 94%, 96%, and 89% respectively. Both of the test products were able to reduce the disease severity. The effect was dose-dependent and it was not related to the moment of application of the fungicides.

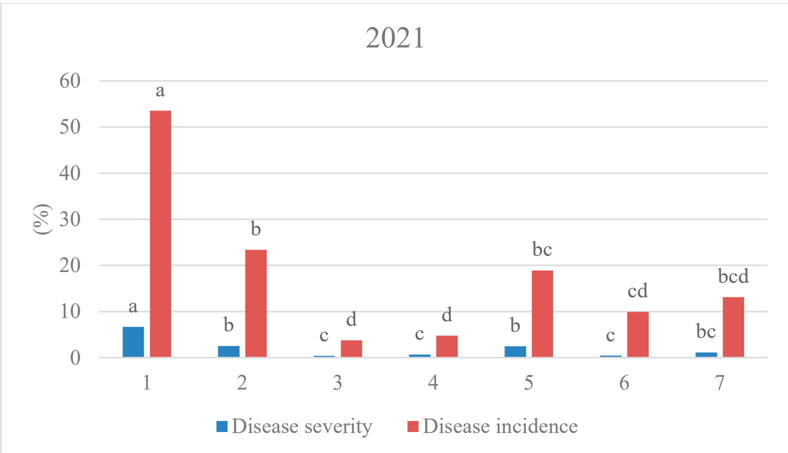

Figure 3. Disease severity and disease incidence of *Fusarium graminearum* in maize, cv. ES Faradi in 2021

The disease incidence was 56% lower than the control when Propulse 250 SE 0.6 l/ha (variant 2) was applied before the inoculation. The reduction in variant 3 was by 93%. The treatment with Prosaro at the same phase (variant 4) led to a 91% decrease in disease incidence. When Propulse 250 SE was applied in a dose of 0.6 l/ha during sporulation (variant 5) the reduction was by 65%. After the application of Propulse 250 SE at a dose rate of

1 l/ha during sporulation (variant 6), the disease incidence was reduced by 81%. The treatment with Prosaro 420 SC at the same phase (variant 7) reduced the disease incidence by 75%.
To explore the effect of the different treatments on the yield, some of its parameters and the content of mycotoxins in grain, namely deoxynivalenol (DON), were analyzed. The yield was harvested on 4 September 2019 during the first experimental year. The analysis of the yield for 2019 is presented in Table 2. The lowest yield was measured in the untreated control. In variant 2, the yield was 1.5% higher; in variant 3-32% higher; in variant 4-3.3% higher, and in variant 5-2% higher than the control. The highest yield was measured in variant 6, where Propulse 250 SE was applied in a dose of 1 l/ha during sporulation (33% higher than the control). The enhancement in variant 7 was by 19% compared to the untreated control. In Table 2, there are also data about moisture content, thousand kernel weight (TKW), hectoliter mass (HLM), and the content of deoxynivalenol (DON) for 2019. It is seen that there are no statistically significant differences in the moisture content of the variants. Regarding the thousand kernel weight, the highest value was measured in variant 3 and variant 4, where Propulse in a dose of 1 l/ha and Prosaro, respectively, before the inoculation, were applied. The lowest values were measured when Propulse was applied during sporulation, regardless of the application dose. The hectoliter mass was the highest in variants 2 and 7 and the lowest - in variant 5. Regarding deoxynivalenol, the highest content was observed in the control plots. In variant 2 (Propulse 250 SE 0.6 l/ha before the inoculation), the reduction of DON was by 73% compared to the control. In variant 5 (Propulse 250 SE 0.6 l/ha during sporulation), the reduction was 65%. In all of the other variants, the content of DON was lower than 100 µg/kg. The accredited laboratory analyses could not detect DON contents below 100 µg/kg.

Table 2. Parameters of the yield and content of DON in maize, cv. DKS5075 in 2019

Variants	Yield (kg/ha)	Moisture content (%)	TKW (g)	HLM (kg)	DON (µg/kg)
1 (Control)	9,015b	10.1	290.1b	77.7c	416
2	9,157ab	9.8	279.1c	78.4a	112
3	11,893ab	9.8	305.6a	77.4e	<100
4	9,320ab	10.3	305.7a	78b	<100
5	9,198ab	9.4	265.3d	75d	147
6	12,029a	9.7	262d	77.6c	<100
7	10,762ab	10.3	290b	78.4a	<100

During the second experimental year, the yield was harvested on 25 September 2020. The data are presented in Table 3. There are no statistically significant differences in the yield quantity and moisture content. Regarding the weight of thousand kernels, the highest value was measured in variant 3 and variant 7, and the lowest was observed in variant 6. There were no statistically significant differences in the hectoliter mass of the variants. The content of DON was the highest in control. In variant 2, DON was reduced by 40% compared to the control. In variant 5, the reduction was by 49%. In all the other treatments, the content of DON was lower than 100 µg/kg.

Table 3. Parameters of the yield and content of DON in maize, cv. SY Phenomen in 2020

Variants	Yield (kg/ha)	Moisture content (%)	TKW (g)	HLM (kg)	DON (µg/kg)
1 (Control)	8,393	8.7	294.8a	74.4	333
2	8,038	8.9	282bc	75.3	201
3	8,460	8.7	299.9a	75	<100
4	8,130	8.5	292.6ab	74.1	<100
5	7,973	9.2	281.8bc	75	169
6	8,488	8.8	277.3c	74.6	<100
7	8,250	8.6	294.5a	75.5	<100

During the third experimental year, the yield was analyzed on 12 September 2021. The data are presented in Table 4. There are no statistically proven differences in relation to the yield and moisture content after the different applications. The TKW was the highest where Propulse 250 SE was applied during sporulation in a dose of 0.6 l/ha (variant 5) and the lowest - in variant 2 and variant 7, where Propulse 250 SE was applied before inoculation in a dose of 0.6 l/ha, and Prosaro during sporulation was applied, respectively. Some differences were also observed in relation to the hectoliter mass. The value was the highest in variant 6 (Propulse 1 l/ha during sporulation) and the lowest - in variant 5 (Propulse 250 SE 0.6 l/ha before inoculation). The DON content was the highest in control, and using Propulse 250 SE in a dose of 0.6 l/ha led to an 85% reduction (variant 2). The same application rate of Propulse 250 SE applied during sporulation led to a decrease of 75% compared to the control. In all of the other variants, the content of DON was lower than 100 µg/kg.

Table 4. Parameters of the yield and content of DON in maize, cv. ES Faradi in 2021

Variants	Yield (kg/ha)	Moisture content (%)	TKW (g)	HLM kg)	DON (µg/kg)
1 (Control)	2,390	9.6	205.9c	62f	834
2	2,115	9.1	201e	62.6e	118
3	2,520	9	211.4b	63.7c	<100
4	2,860	9.8	207.5	63.6d	<100
5	2,755	9.3	212.3a	61.4g	209
6	2,838	8.7	203.7d	64.7a	<100
7	2,465	9.1	201.7e	64.3b	<100

According to Andersen et al. (2017), Prothioconazole was able to reduce the *Gibberella* ear rot severity but did not reduce the content of DON in maize. Regarding the disease severity, these announcements align with our findings. On the other hand, our results are the opposite concerning the DON content. The research of Limay-Rios & Schaafsma (2018) announced that the early application of prothioconazole reduced the content of DON in maize. The authors observed no reduction in *Fusarium graminearum* toxins when the fungicide was applied after the silk senescence stage. Our study confirms that the fungicidal treatment was able to reduce the mycotoxin content. It was established that all the test products reduced the content of deoxynivalenol, and the effect was dose-related.

CONCLUSIONS

The results of the present study showed that using chemical pesticides could reduce the severity and incidence of *Gibberella* ear rot (*Fusarium graminearum)* on maize and the content of deoxynivalenol in the yield. Most of the results in the three experimental years were characterized by a similar tendency. The effect of the applications was dose and, in some cases, time-depended. Both of the experimental doses of Propulse 250 SE reduced the disease incidence and severity, but better results were achieved when the higher dose (1 l/ha) of Propulse 250 SE was applied. The content of mycotoxins was dramatically reduced when Propulse 250 SE in a dose of 1 l/ha and Prosaro 420 were applied. Regarding the moment of application, the treatments before inoculation had a better effect on mycotoxin reduction in 2019 and 2021. In 2020, the DON content was lower when Propulse 250 SE was applied during sporulation. Regarding the yield, applying Propulse 250 SE in a dose of 1 l/ha during sporulation in the first experimental year led to a statistically significant increase of 33% compared to the untreated control. For the second and third experimental years, the differences were not statistically proven.

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PRODUCTIVITY OF DURUM WHEAT VARIETIES IN THE SOIL AND CLIMATE CONDITIONS OF PLOVDIV REGION

Tanko KOLEV, Zhivkpo TODOROV

Agricultural University of Plovdiv, 12 Mendeleev Blvd, Plovdiv, Bulgaria

Corresponding author email: tanko_kolev@yahoo.co.uk

Abstract

Field experience was carried out in 2017-2020 at the Educational Experimental and Implementation Base of the Agricultural University of Plovdiv. The following new varieties of Durum wheat selected at the Institute of Field Crops in the city of Chirpan were tested: Kehlibar, Reyadur, Tserera and Trakiets. They are compared to the standard of productivity variety Predel. The aim of the study is to establish the productivity of Durum wheat variety Kehlibar, Reyadur, Tserera and Trakiets under the soil and climatic conditions of the Plovdiv region. The field experiment was conducted using the block method in four replications with a harvest plot size of 15 m^2. As a result of the three-year study, it was found that the productivity of Durum wheat variety Reyadur was the highest 4.520 t/ha with 0.455 t/ha (11.2%) higher grain yield compared to the standard variety Predel. Followed by Kehlibar variety 4.328 t/ha with 0.263 t/ha (6.5%); variety Trakiets 4.209 t/ha with 0.144 t/ha (3.5 %) and variety Tserera 4.146 t/ha with 0.081 t/ha (2.0 %) more grain compared to variety Predel. The higher productivity of the new varieties of Durum wheat is the result of the increased values of the structural components of the grain yield: productive tillering, number of grains and weight of grains per ear.

Key words: *durum wheat, varieties, productivity, grains yield.*

INTRODUCTION

In recent years, the interest in durum wheat has been growing, and the areas and average yields from it are constantly expanding. During the past ten years, durum wheat occupies 13 to 16 million hectares of land globally, with a trend to increase the area.

Durum wheat is grown in the world on 13.5 million ha in 2020/2021, which represents 6.2% of the wheat area (Martínez-Moreno et al., 2022.)

Durum wheat in Europe in 2021 occupies an area of 2348000 ha, with yield 3230 kg/ha, and production 7581000 t.

Global demand for durum wheat is expected to grow by 1.0 MMT (Million Metric Tons) to 32.9 MMT in 2022/23. As a result of lower domestic production in many countries, world trade in durum wheat is estimated to grow by 1.5 MMT to 7.4 MMT in 2022/23.

The strategy Grains forecasts that EU import of durum wheat from non-EU members to be 2.4 MMT, i.e. with 1.0 MMT more than last year. Morocco's imported quantity is expected to increase by 400,000 MT to 1.1 MMT.

The necessity to increase crop production poses major tasks that must also be taken into account when solving the grain problem, including and in the production of grain from durum wheat (*Triticum durum* Desf.). In this respect, the importance of variety and variety structure are crucial (Alvaro, 2008; Arduini et al., 2006; Vafa et al., 2014; Bilgin et al., 2008; Sabella, 2020; Dekov et al., 1989; Kodanev, 1970; Sozinov, 1983).

Bulgaria is small in terms of territory, but very diverse in terms of soil and climatic conditions. To obtain high and stable yields and good revenue from durum wheat, the selection of a suitable variety composition for the respective ecological region is essential for the eventual economic results. This is required by the global changes that have occurred in the climate in recent years and the adaptive abilities of varieties to these conditions.

The purpose of the research carried out is to establish the productive abilities of new varieties of durum wheat in the soil and climatic conditions of Plovdiv region.

MATERIALS AND METHODS

The field experiment was carried out during the period 2017-2020 in the Educational Experimental and Implementation Unit of the

Agricultural University of Plovdiv using the block method, in four repetitions and the size of the harvest plot of 15 m^2. The following new varieties of durum wheat (*Triticum durum* Desf.) selected at the Institute of Field Crops in Chirpan were tested: Kehlibar, Reyadur, Cerera and Trakiets. They are compared to the standard of productivity, i.e. Predel variety.

The soil on which the field experiment was conducted is carbonate alluvial-meadow soil Molic Fluvisols (FAO - UNESCO, 1990), which is characterized by an average sandy-clay mechanical composition, humus content 1-2%. The soil is characterized by a slightly alkaline pH reaction (7.2-7.7), the presence of carbonates (4.3-7.4%) and the absence of salts. In the soil layer of 0-20 cm, the content of the main nutritional elements was as follows: N - 15.1 mg/1000 g; P_2O_5 - 30 mg/100 g; K_2O - 45 mg/100 g (Popova & Sevov, 2010). The soil has good physico-mechanical properties, loose structure, low plasticity and stickiness with good moisture capacity and filtration ability. (Tahsin & Popova, 2005).

The generally accepted technology was complied with in the cultivation of durum wheat (Bozhanova et al., 2018). The seeds of Durum wheat was sown in the optimal period from 20.10. to 5.11. in each year of experiment, with a seeding rate of 500 germinating seeds/m^2 and mineral fertilization 80 kg/ha P_2O_5 and 140 kg/ha N as active substance, as the entire amount of phosphorus fertilizer and 1/3 of the nitrogen fertilizer were applied before sowing, and the remaining quantity of the nitrogen fertilizer was applied in early spring as an additional nutrition.

The structural elements of the yield were recorded: productive tillering, length of the wheat-ear (cm), number of grains in the wheat-ear, mass of the grains in the wheat-ear (g), and from the physical indicators of the grain - mass of 1000 grains (g), hectolitre mass (kg/hl) and vitreousness of the grain (%). Grain yield (t/ha) was reported by variants and repetitions. The harvest was carried out at full maturity, by direct harvesting with a small trial harvester Wintersteiger seedmaster universal.

The grain samples taken from the tested varieties of durum wheat were qualified according to the following indicators: mass per 1000 grains according to BDS ISO 520:2003; hectolitre mass according to BDS ISO 7971-2:2000; glassy quality of the grain according to BDS EN 15585:2008 in the Accredited Laboratory Complex for testing at the Agricultural University of Plovdiv.

The statistical processing of the data obtained on the studied indicators was carried out with the BIOSTAT software (Penchev, 1998).

RESULTS AND DISCUSSIONS

During the period of the field experiment (2017-2020) during the vegetation of durum wheat, higher average monthly temperatures were observed compared to the climatic standard.

More significant deviations from the standard were observed in the precipitation during the durum wheat vegetation. The amount of raifall is as follows: 2017-2018 - 457.2 mm, in 2018-2019 - 466.1 mm and in 2019-2020 - 478.9 mm against 419.6 mm for the climatic standard.

The amount of rainfall in the three experimental years exceeds the climatic norm and due to its better distribution during the critical stages of plant development, the 2018-2019 harvest is more favorable for the growth and development of durum wheat. Due to the higher amount of precipitation during the flowering period in the months of April and May, respectively by 44 mm/m^2 and by 15.4 mm/m^2 more compared to a multi-year period, the pollination and fertilization of the flowers did not proceed normally, which also led to the formation of lower number of grains and lower grain yield in 2020 (Figures 1 and 2).

Average daily temperatures during the three years of the experiment were as high as the multi-year period. The exception is the month of April 2020, when they are lower compared to the climatic norm.

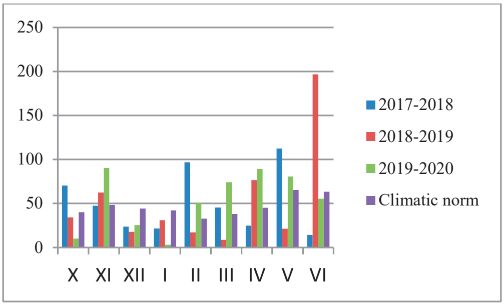

Figure 1. Raifall by months, sum mm/m^2, in Plovdiv region

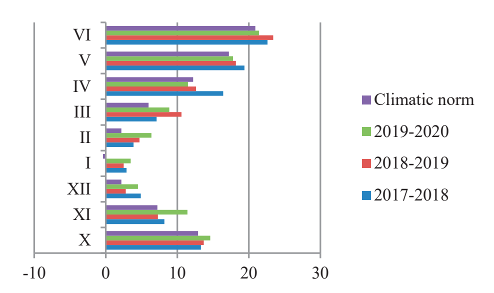

Figure 2. Monthly temperatures (average), in Plovdiv region

Biometric data of the studied varieties of durum wheat

Plant height. Plant height is primarily a characteristic of the variety (Zhelev, 1980; Dekov, 1982) and is a relatively constant value, but soil and climatic conditions and growing technology have a significant impact on the values of this indicator. The amount of rainfall in the spring, and more precisely from the tillering phase to the end of wheat-ear formation, significantly affects the height of the plants (Table 1[a]).

Table 1[a]. Biometric data (average 2017-2020)

Variety	Height of the plants, cm	Productive tillering, number	Wheat-ear length, cm
Predel	86.7	1.38	7.93
Kehlibar	88.2	1.55	8.22
Reyadur	89.5	1.72	8.45
Tserera	87.1	1.41	7.99
Trakiets	86.9	1.47	8.03
GD 5 %	2.76	0.16	0.25

In the carrying out of the present experiment, it was found that the plants with the highest stem were of the Reyadur variety - 89.5 cm (3.2%) on average during the research period which is higher than the Predel variety (Table 1[a]). Higher than the standard variety Predel are the varieties Trakiets (by 0.2 cm), Tserera (by 0.4 cm) and Kehlibar (by 1.5 %).

Productive tillering. The tillering of durum wheat depends on the variety, soil and climatic conditions, seeding density, seeding depth, availability of nutrients, light, time of sowing, seed size (Spaldan et al., 1984). Tillering is highly correlated with yield formation (Yani et al., 2012; Elhani et al., 2007; Delchev et al., 2000).

The ratio between the number of formed ears per unit area to the number of maximally formed tillers determines the productive tillering.

The highest productive tillering is demonstrated for the following varieties: Reyadur - 1.72 tillers and the Kehlibar variety - 1.55 tillers, respectively with 0.34 tillers and 0.17 tillers more than the standard Perdel variety. Then follow the varieties - Trakiets - 1.47 and Tserera - 1.41, which is by 0.09 tillers and by 0.03 tillers more than the standard (Table 1[a]).

Wheat-ear length is a genetically determined trait for each durum wheat variety and as such it is a relatively constant value (Yanev et al., 2000).

On average for the research period, the longest ears were formed by the plants of the Reyadur variety 8.45 cm (6.6%), followed by the Kehlibar variety 8.22 cm (3.7%), Trakiets 8.03 cm (1.3%), Tserera 7.99 cm (0.8%) (Table 1[a]).

Number of spikelets in a wheat-ear. Ear density depends on ear length and the number of spikelets per unit length. Climatic conditions, as well as the application of appropriate technology in the cultivation of durum wheat, play an essential role in the formation of spikelets in the ear.

During the experiment, we found that the Reyadur variety has the largest number of spikelets in the year, 29.3 pcs. it was followed by the Kehlibar variety with 27.9 pcs., Trakiets with 26.4 pcs. and Tserera with 25.1 pcs. which is respectively by 18.1 %, 12.5 %, 6.5 % and 1.2 % spikelets more than the standard (Table 1[b]).

Number of grains per wheat-ear. One of the important elements of productivity in cereal crops is the number of grains in the ear. By applying the optimal technological measures, their increase is achieved, which leads to an increase in grain yield per hectare.

Rainfall in the spring has a significant influence on the number of grains, and more precisely from the tillering phase to the end of the flowering phase. In the months of April and May of the third year of the study, the amount of precipitation was by 44 mm/m^2 and 15.4 mm/m^2, respectively, more compared to a multi-year period. Due to the higher amount of precipitation during flowering, the normal pollination and fertilization of the flowers did not take place, which also led to the formation

of a smaller number of grains and a lower grain yield in 2020.
It was established that, on average, during the three-year period of the experiment, the Reyadur variety formed the largest number of grains in the ear, 52.7 pcs. (8.7%). In second place is the Kehlibar variety 51.3 pcs. (5.8%), Trakiets variety with 49.4 pcs. (1.9%) and variety Tserera 48.7 pcs. (0.4%) compared to the standard variety Predel (Table 1^b).

Table 1^b. Biometric data (average 2017-2020)

Variety	Number of spikelets in a wheat-ear	Number of grains per wheat-ear	Mass of grains in the wheat-ear
Predel	24.8	48.5	2.14
Kehlibar	27.9	51.3	2.27
Reyadur	29.3	52.7	2.38
Tserera	25.1	48.7	2.19
Trakiets	26.4	49.4	2.21
GD 5 %	2.84	2.52	0.12

Mass of grains in the wheat-ear is a very important component for the productivity of durum wheat. Several factors play an important role for the value of this indicator, such as: weather conditions during the period of grain formation, as well as the later development of plants until the end of the vegetation, cultivation technology, as well as the genetic abilities of a given variety.
In the implementation of the three-year field experiment, it was found that of all tested durum wheat varieties, the highest grain mass in the ear was demonstrated by the plants of the Reyadur variety - 2.38 g (11.2 %) (Table 1^b). It was followed by the varieties Kehlibar 2.27 g (6.1 %), Trakiets with 2.21 g (3.3 %), Tserera with 2.19 g (2.3 %) (Table 1^b).
Grain yield. The interaction between the soil and climatic conditions in the durum wheat production area and the studied variety have a significant impact on the yield and quality of the obtained grain.
The tested new varieties of durum wheat selected at the Institute of Plovdiv exceed in terms of grain yield the productivity standard Predel variety (Table 2).
The interaction between the soil and climatic conditions in the durum wheat production area and the studied variety have a significant impact on the yield and quality of the obtained grain.

Table 2. Grain yield the productivity

Variety	2018 t/ha	2019 t/ha	2020 t/ha	Average	
				kg/ha	%
Predel	4.150	4.571	3.473	4.065	100.0
Kehlibar	4.384	4.975	3.624	4.328	106.5
Reyadur	4.753	5.054	3.778	4.520	111.2
Tserera	4.215	4.632	3.591	4.146	102.0
Trakiets	4.295	4.843	3.569	4.236	104.2
GD 5 %	0.230	0.267	0.149		

The tested new varieties of durum wheat selected at the Institute of Field Crops in Plovdiv exceed in terms of grain yield the productivity standard Predel variety (Table 2). For all tested varieties, the highest amount of grain was obtained in the climatically favorable year for the growth and development of durum wheat which is 2019, followed by that of 2018. The grain obtained of the tested varieties was the least in 2020, due to the higher quantity of precipitation during flowering, which led to difficulties in pollination and fertilization of the flowers, and therefore to the formation of a smaller number of grains and a lower grain yield.
From the data presented in Table 2, it is clear that both by year and on average for the research period, the highest grain yield is obtained from the Reyadur variety - 4.520 t/ha (11.4%) compared to 4,065 t/ha for the standard Predel variety. By years, in 2018 -4.753 t/ha (14.5%), in 2019 - 5.054 t/ha (10.6 %) and in 2020 - 3.778 t/ha (8.8%) were obtained from the Reyadur variety. The increase in grain yield by year is respectively 0.603 t/ha - in the first year, 0.483 t/ha in the second and 0.305 t/ha in the third, or on average by 0.455 t/ha more than the productivity standard Predel variety.
The results are one-way and mathematically very well proven.
From the varieties Kehlibar and Trakiets, on average, during the experimental period, 4.328 t/ha (6.5%) and 4.236 t/ha (4.2%) were obtained, respectively, which is 0.263 t/ha and 0.171 t/ha more than the Predel variety. The higher productivity of the Kehlibar variety was mathematically proven during the three years of the experiment, and the Trakiets variety proved a higher yield only in 2019.
The increase in grain yield in the Tserera variety is mathematically unproven.

Physical properties of durum wheat varieties

Mass of 1000 grains. The mass of 1000 grains is an indicator that characterizes the grain's fullness and serves as an indicator of determination of output. Large-sized grains are used as sowing material and yield from them is up to 15-20% higher (Dekov et al., 1989). The mass of 1,000 grains is a varietal characteristic, which is strongly influenced by soil and climatic conditions and cultivation technology.

Of the studied varieties of durum wheat Kehlibar variety stands out with the highest mass of 1000 grains (Table 3). Average for the study period, the weight of 1000 grains in this variety was 52.4 g, followed by the Reyadur variety with 51.8 g. For Trakiets variety it is slightly higher than the standard, and for the Tserera variety the mass of 1000 grains is lower than the standard (Table 3).

Table 3. Physical properties of durum wheat varieties

Variety	Mass of 1000 grains, g	Hectolitre mass, kg/hl	Vitreousness of the grain, %
Predel	50.1	80.15	96.2
Kehlibar	52.4	82.60	98.5
Reyadur	51.8	81.21	97.8
Tserera	49.5	80.53	97.1
Trakiets	50.7	80.72	96.7
GD 5 %	1.35	1.02	1.46

Hectoliter mass. An important physical indicator that serves to determine grain quality is the hectoliter mass. It gives an idea of uniformity, surface and density. The hectoliter mass depends on the type of impurities, the weed seeds, the unthreshed ears and the consistency of the grain.

Of the studied varieties of durum wheat, with a higher hectoriter mass of the grain are the Kehlibar variety - 82.60 kg/hl and Reyadur variety - 81.21 kg/hl compared to the Predel variety (Table 3).

Vitreousness of the grain is an important physical property and its high values determine the production of high-yield flours. This indicator is strongly influenced by many factors, but the decisive ones are the variety with its characteristics, the soil and climatic conditions during the formation and ripening of the grain.

Durum wheat is characterized by a high glassy grain. It can be seen from Table 3 that, on average, for the three-year experimental period, the glassy quality was very high for all studied varieties of durum wheat. For the Kehlibar variety it is 98.5%, followed by Reyadur with 97.8%, Tserera with 97.1%, Trakiets with 96.7%, and for the standard Predel variety - it is 96.2%.

CONCLUSIONS

The highest productivity in the soil and climatic conditions of Plovdiv region was reported for the Reyadur durum wheat. The harvested grainof this variety is in the range of 4.753 t/ha (14.5%) in 2018, of 5.054 t/ha (10.6%) in 2019 and 3.778 t/ha (8.8%) in 2020. The increase of grain yield by year is respectively 0.603 t/ha – in the first year, 0.483 t/ha in the second and 0.305 t/ha in the third, or an average of 0.455 t/ha more that the productivity standard Predel variety.

Kehlibar and Trakiets on average, during the experimental period, 4.328 t/ha (6.5%) and 4.236 t/ha (4.2%) were obtained, respectively, which is 0.263 t/ha and 0.171 t/ha more than Predel variety.

The productive abilities of the studied new varieties of durum wheat are greater compared to the standard Predel variety, which is a result of the higher productive tillering, the longer and more grain-filled ear and the higher grain mass in the plant ears.

The durum wheat variety Kehlibar has the highest mass per 1000 grains on average for the study period, which is is 52.4 g, followed by the Reyadur variety with 51.8 g. Trakiets variety has slightly higher values than the standard, and Tserera variety's mass per 1000 grains is lower than the standard Predel variety.

The hectoliter mass of the grain of the studied durum wheat varieties is greater in the varieties Kehlibar 82.60 kg/hl and Reyadur 81.21 kg/hl compared to Predel variety.

The glassy quality in the new tested durum wheat cultivars was very high on average over the three-year study period. For Kehlibar variety it is 98.5%, followed by Reyadur with 97.8%, Tserera with 97.1%, Trakiets with 96.7%, while for the Predel standard it is 96.2%.

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THE EFFICIENCY OF THE APPLICATION OF PREPARATIONS BASED ON ADHESIVE AND SURFACTANT SUBSTANCES AND DESICCANTS IN GROWING SESAME SEEDS

Vira KONOVALOVA[1], Volodymyr KONOVALOV[2], Andrii TYSHCHENKO[1], Olena TYSHCHENKO[1], Serhii ROI[1], Nadiia REZNYCHENKO[1], Viktor SHARII[1], Kateryna FUNDIRAT[1]

[1]Institute of Climate-Smart Agriculture of the National Academy of Agrarian Sciences of Ukraine, 24 Mayatska Doroga Street, sett. Khlybodarske, Odesa District, Odesa Region, 67667, Ukraine
[2]Askanian State Agricultural Research Station of the Institute of Climate-Smart Agriculture of the National Academy of Agrarian Sciences of Ukraine, Tavrichanka Village, Kakhovka District, Kherson Region, 74862, Ukraine

Corresponding author email: tischenko_andriy@ukr.net

Abstract

The purpose of the study was to establish the use of various desiccants, preparations based on adhesives and surface-active substances and their combination on the productivity and seed preservation of sesame plants. The research was conducted under irrigation in the arid climatic zone of the Southern Steppe of Ukraine. In these studies, the best option for the use of various desiccants and preparations based on adhesives and surface-active substances was established. Sesame seed productivity and yield structure elements were established; correlation analysis was conducted.

***Key words**: sesame, seeds, desiccant, adhesives and surfactants, yield.*

INTRODUCTION

Sesame (*Sesamum indicum* L.) (2n = 26) is one of the oldest and early-ripening oil crops in the world. Belongs to the order Tubiflorae, family Pedaliaceae, genus Sesamum, and is the most commonly cultivated species of oilseed out of more than 30 species in this genus (Zhang et al., 2013). Predominantly considered a self-pollinated plant, although a low percentage of cross-pollination has been reported (Ashri, 2007).

The world sown area of sesame is about 10.5 million hectares with an annual production volume of about 6.0 million tons (Sharaby & Butovchenko, 2019). The average yield varies from 300 to 500 kg/ha, but with proper care and the use of modern agricultural technologies, it can reach 3000 kg/ha (Abadi, 2018). The world's leading countries in sesame production are India, Myanmar, China, Sudan, Tanzania, Ethiopia, Uganda and Nigeria. Japan is the largest importer of sesame in the world due to sesame oil being an important ingredient in Japanese food, followed by China, which is the second largest importer of sesame in the world, although it is one of the largest producers of sesame seeds. In addition, there are many other major sesame importing countries such as USA, Netherlands, Turkey, Canada as well as France. Recently, the consumption of products and oil from sesame seeds has been steadily increasing both in Europe and the USA (Abate, 2015).

Sesame is widely known as the king of oil crops due to the high content of oil in the seeds (50-63%), 17-32% protein (rich in sulfur containing amino acids), and 80% of sesame oil consists of unsaturated fatty acids, (Eskandari et al., 2015), it is grown for food, medical purposes or used for biodiesel production (De Lima et al., 2020). The seeds are highly stable and resistant to rancidity and are used in pasta, candies, pies, paints, soaps, cosmetics and medicines, as well as ingredients in breads, crisps and health foods (Dias et al., 2017). Sesame has high therapeutic and nutritional value (Anastasi et al., 2017), and has been recognized as a good source of high-quality oil with a high proportion of unsaturated fatty acids, proteins and antioxidants (Bahrami et al., 2012). In addition, sesame seeds are rich in minerals (calcium, iron, phosphorus) and vitamins (vitamin A, thiamin and riboflavin). Due to its high quality, sesame is also called the "queen of oil crops" (Deepthi et al., 2014). Sesame oil is highly valued for its nutritional value associated with health benefits, and the quality index (ratio of unsaturated fatty acids to saturated fatty acids) for edible oil in sesame varies from 83-87% in the seed (Wei et al., 2015; Eskandari et al., 2015).

The consumption of sesame seeds or oil has numerous pharmacological properties, such as lowering blood lipids and arachidonic acid levels, lowering cholesterol (Visavadiya & Narasimhacharya, 2008), providing antiproliferative activity (Yokota et al., 2007) and anti-inflammatory function (Hsu et al., 2005), increasing fatty acid oxidation enzymes in the liver, showing antihypertensive (Nakano et al., 2008) and neuroprotective effects against hypoxia or brain damage (Cheng et al., 2006). Currently, sesamin is the most powerful food known to effectively improve the bioavailability of γ-tocopherol (Cooney et al., 2001). Such characteristics have expanded the use of sesame in antiseptics, bactericides, functional food products, pharmaceutical and cosmetic preparations (Namiki, 2007).

Sesame is a drought-resistant crop (Tewelde, 2019). Studies conducted on sesame in different countries have shown that it prefers high temperatures and limited soil moisture to obtain satisfactory yields (Bahrami et al., 2012).

However, in recent decades, climate changes have been observed, the so-called "global warming", as a result of which the temperature regime is increasing, dry periods are becoming more frequent and their duration is increasing (Vozhehova et al., 2022c; Tyshchenko et al., 2020b). This significantly affects the amount of precipitation and its redistribution during the growing season and is one of the main abiotic stress factors, which leads to a significant decrease in the yield of agricultural crops (Tyshchenko et al., 2020a; Vozhehova et al., 2021b). However, in recent times, during the ripening period of late agricultural crops, which includes sesame, frequent rains with showers are observed, which increases the moisture content of seeds, prevents timely harvesting and, accordingly, leads to crop losses (Vozhehova et al., 2022a). Rainy weather combined with strong winds at maturity negatively affects the yield of sesame due to the cracking of pods and the spilling of seeds. Seed shedding is a serious cause of crop losses (Abadi, 2018; Dissanayake et al., 2017).

Therefore, we decided to conduct research on seed preservation using surface-active and adhesive substances and desiccants.

The purpose of the work. Investigate the effect of surface-active and adhesive substances and desiccants on the preservation and productivity of sesame seeds.

MATERIALS AND METHODS

The research was conducted during 2019-2021 at the experimental field of the Askanian State Agricultural Research Station in the village of Tavrychanka, Kherson region (46°33'12"N; 33°49'13"E; 39 m above sea level) of the Institute of Climate-Smart Agriculture of the National Academy of Agricultural Sciences of Ukraine. In terms of soil and climate, it is located in the steppe zone, on the Kakhovsky irrigated massif.

Study 1. A one-factor field experiment on the study of different desiccants (Preparation 1 - 150 g/l ammonium glufosinate - 2.0 l/ha, Preparation 2 - 150 g/l ion diquat - 3.0 l/ha and Preparation 3 - 450 g/l glyphosate in acid equivalent (551 g/l in the form of the potassium salt of glyphosate) - 2.4 l/ha) and their use in different stages of pod browning (70-75% and 80-85%) for the preservation and yield of sesame seeds.

Study 2. The two-factor field experiment is based on the method of split plots. Main areas (factor A) - application of preparations based on adhesives and surface-active substances (Raps kley with a consumption rate of 1.2 l/ha, Agrolip - 1.5 l/ha and N′yu Fylm -17-1.0 l/ha) n the browning phase of pods 70-75%; sub-sites (factor B) - application of preparations based on adhesives and surface-active substances (Raps kley - 1.2 l/ha, Agrolip - 1.5 l/ha and N′yu Fylm – 17 - 1.0 l/ha) n combination with a desiccant with active with the substance 150 g/l diquat ion - 3.0 l/ha was carried out in the browning phase of the pods 80-85%

The experiments were based on irrigation, watering was carried out with a sprinkler and maintenance of soil moisture at the level of 75-80% of the lowest moisture content. Sesame variety Husar. Wide-row sowing with 70 cm between rows. The area of the sowing area is 60 m^2, the accounting area is 50 m^2, repetition three times. The consumption of working fluid during processing was 250 l/ha.

Statistical processing of experimental data was carried out by AgroSTAT, XLSTAT, Statistica (v. 13).

RESULTS AND DISCUSSIONS

According to the results of previous studies, the indicators that determine the amount of sesame yield are the number of pods and seeds on one plant, as well as the weight of seeds from one plant. Each of these elements can vary greatly depending on agronomic practices leading to increased or decreased yields, especially the number of pods and seeds retained at harvest.

On the control version (without the use of a desiccant), the smallest number of 4732 seeds per plant, seed weight of 12.73 g per plant, and seed yield of 0.85 t/ha were recorded during harvesting. However, the mass of 1000 seeds was the largest - 2.69 g. When desiccants were used in the 70-75% phase, browning of the pods contributed to obtaining the largest number of 5264-5910 seeds per plant, seed mass of 13.32-15.37 g per plant, and yield seeds 1.02-1.18 t/ha. However, the mass of 1,000 seeds was the smallest - 2.53-2.61 g. The best result when processed in the phase of 70-75% browning of pods was shown by Preparation 2 with a seed yield of 1.18 t/ha, which was higher than the control variant by 0.33 t/ha (Table 1).

Table 1. Elements of the yield structure and seed productivity of sesame depending on the application of desiccants

Variants of the experiment	Number of pods on 1 plant (n_c), pcs.	Number of seeds on 1 plant (n_s), pcs.	Seed weight from 1 plant (m_s), g	Weight of 1000 seeds (m_{1000}), g	Yield (Y), tons/ha	Yield increase relative to control, t/ha	Average yield by desiccants, t/ha
Control (without treatment)	91	4732	12.73	2.69	0.85	-	-
Preparation 1 (with browning 70-75%)	88	5632	14.70	2.61	1.02	0.17	0.96
Preparation 2 (with browning 70-75%)	93	5910	15.37	2.60	1.18	0.33	1.07
Preparation 3 (with browning 70-75%)	94	5264	13.32	2.53	1.11	0.26	1.03
Preparation 1 (with browning 80-85%)	92	4804	12.73	2.65	0.89	0.04	
Preparation 2 (with browning 80-85%)	90	5045	13.27	2.63	0.96	0.11	
Preparation 3 (with browning 80-85%)	89	4987	12.92	2.59	0.94	0.09	
LSD_{05}	1.01	15.2	0.2	0.03	0.02		

The use of desiccants in the phase of 80-85% browning of pods increased the weight of 1000 seeds by 0.03-0.04 g, but decreased the weight of seeds per plant to 0.40-2.10 g and, accordingly, the yield of sesame seeds by 0.13-0.22 t/ha. Of the three desiccants when processed in the phase of 80-85% browning of the pods, the highest yield of 0.96 t/ha was obtained with the use of Preparation 2.

The correlation analysis between the elements of the crop structure and the seed productivity of sesame plants was carried out. The correlation between the number of pods on one plant and seed yield was 0.442, between the number of seeds on one plant and seed yield was 0.890, the weight of seeds on one plant and seed yield was 0.795, and the weight of 1000 seeds and seed yield was -0.738 (Figure 1).

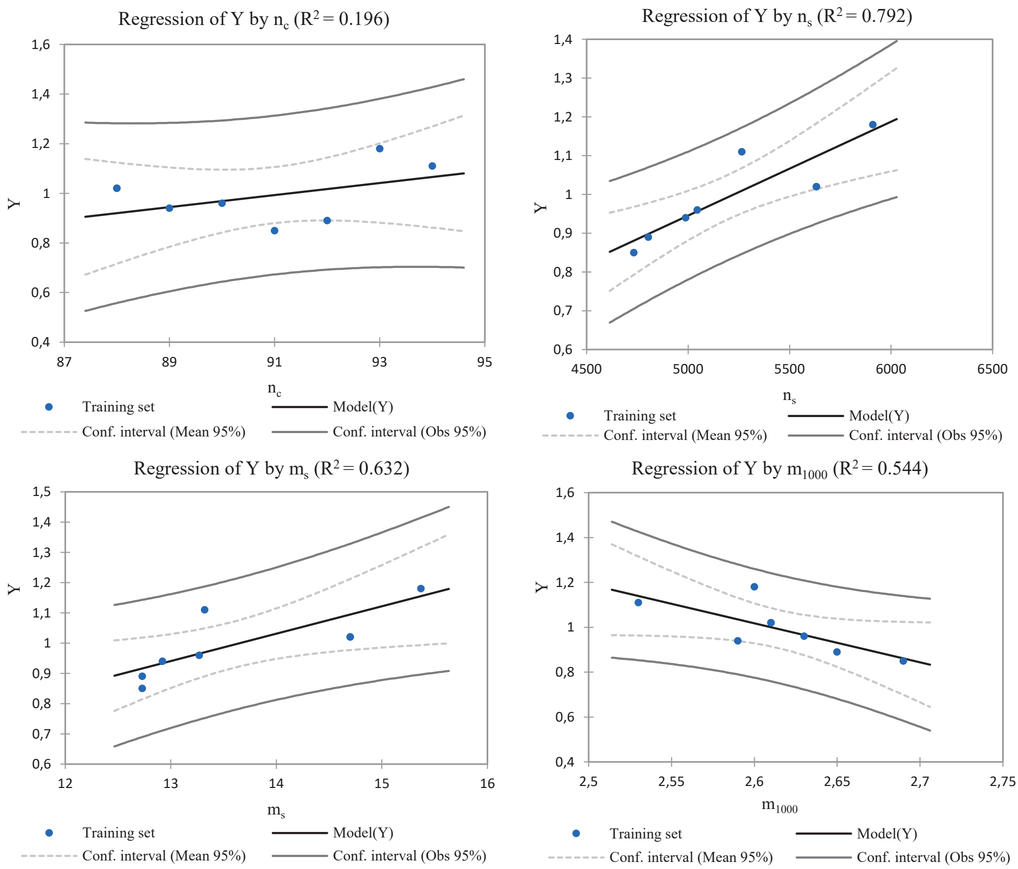

Figure 1. Regression diagrams of the number of pods per 1 plant (n_c) and seed yield (Y) (top left), the number of seeds per 1 plant (n_s) and seed yield (Y) (top right), seed weight per 1 plant (m_s) and seed yield (Y) (bottom left), weight of 1000 seeds (m_{1000}) and seed yield (Y) (bottom left)

The inverse relationship between the weight of 1000 seeds and seed yield is explained by the fact that the weight of 1000 seeds is greater in the control variant than in the variants with the use of desiccants, and the yield is lower. The same is observed on the options when treated with desiccant in the phase of 80–85% browning of the pods, compared to the treatment in the phase of 70-75%. That is, on the control variant, or on the variants treated with desiccant in the phase of 80-85%, the browning of the seed pods was more complete and was characterized by a higher mass of 1000 seeds.

Uneven ripening of sesame pods is a biological feature of the crop that leads to seed loss. According to our research, the use of preparations based on adhesives and surface-active substances prevents the premature cracking of pods, increases the yield and improves its quality, is not washed away by rain, reduces pre-harvest losses of the crop and allows collecting uniform ripe seeds.

As a result of the research, the smallest number of seeds was 3080 pcs. from one plant, seed mass of 8.75 g from one plant and seed productivity of 0.81 t/ha were obtained on the control variant (without treatments). During the first treatment with preparations based on adhesives and surface-active substances in the phase of 70-75% browning of the pods, an increase in the number of seeds by 245-461 pcs/plant, the weight of seeds per plant by 0.69-1.52 g, and seed productivity is observed by 0.05-0.12 t/ha compared to the control variant. The largest number of seeds of 3541 per 1 plant, seed weight per plant of 10.27 g and seed productivity of 0.93 t/ha during the first

treatment was obtained with the use of Agrolip (Table 2).
During the second treatment with a desiccant with the active substance 150 g/l diquat ion in the phase of 80-85% browning of the pods, an increase in the number of seeds by 165 pcs/plant, seed weight per plant by 0.04 g, and seed productivity by 0.07 t was observed /ha with a decrease in the weight of 1000 seeds by 0.13 g compared to the control variant. While treatment with preparations based on adhesives and surface-active substances in combination with a desiccant increases the number of seeds by 265-404 pcs/plant, the weight of seeds per plant by 0.52-1.04 g, and seed productivity by 0.12-0.17 t/ha with a decrease in the weight of 1000 seeds by 0.03-0.07 g compared to the control variant.
That is, the use of a desiccant contributes to uniform drying of the herbage, ripening, improved harvesting, but its use does not prevent crop loss. Therefore, the use of preparations based on adhesives and surface-active substances in the first treatment, and their combination with a desiccant in the second, allows to reduce crop losses.
The best option turned out to be when using Agrolip in the first treatment and Desiccant + Agrolip in the second. On this variant, 4,085 seeds were formed per 1 plant, the weight of seeds from one plant was 11.72 g, and the seed productivity was 1.14 t/ha with a weight of 1,000 seeds - 2.87 g.
A somewhat lower seed productivity of 1.13 t/ha, the number of seeds 3887 per 1 plant, the weight of seeds from one plant 11.04 g with the weight of 1000 seeds - 2.84 g was characterized by the variant when applied in the first treatment of N′yu Fylm-17 and second - Desiccant + Agrolip.

Table 2. The influence of the use of preparations based on adhesives and surface-active substances, desiccants on productivity elements and seed productivity of sesame plants

First processing (when browning pods 70–75%)	Second processing (when browning pods 80–85%)	Number of pods on 1 plant (n_c), pcs.	Number of seeds on 1 plant (n_s), pcs.	Weight of seeds from 1 plant (m_s), g	Weight of 1000 seeds (m_{1000}), g	Yield (Y), tons/ha	Average yield after the second processing, t/ha
Control (without treatments)	Control (without treatments)	70	3080	8.75	2.84	0.81	0.81
	Desiccant	72	3245	8.79	2.71	0.89	0.95
	Desiccant + Raps kley	71	3345	9.27	2.77	0.93	1.00
	Desiccant + Agrolip	70	3484	9.79	2.81	0.98	1.07
	Desiccant + N′yu Fylm-17	74	3389	9.46	2.79	0.97	1.03
	Average	**71**	**3309**	**9.21**	**2.78**	**0.92**	
Raps kley	Control (without treatments)	70	3325	9.44	2.84	0.86	
	Desiccant	72	3452	9.53	2.76	0.90	
	Desiccant + Raps kley	71	3554	9.95	2.80	0.95	
	Desiccant + Agrolip	69	3672	10,.36	2.82	1.01	
	Desiccant + N′yu Fylm-17	70	3586	10.08	2.81	0.98	
	Average	**70**	**3518**	**9.87**	**2.81**	**0.94**	
Agrolip	Control (without treatments)	71	3541	10.27	2.90	0.93	
	Desiccant	69	3682	10.31	2.80	1.02	
	Desiccant + Raps kley	72	3854	10.87	2.82	1.07	
	Desiccant + Agrolip	74	4085	11.72	2.87	1.14	
	Desiccant + N′yu Fylm-17	72	3996	11.39	2.85	1.09	
	Average	**72**	**3832**	**10.91**	**2.85**	**1.05**	
N′yu Fylm-17	Control (without treatments)	72	3474	9.94	2.86	0.89	
	Desiccant	70	3598	10.00	2.78	0.97	
	Desiccant + Raps kley	73	3698	10.35	2.80	1.05	
	Desiccant + Agrolip	71	3887	11.04	2.84	1.13	
	Desiccant + N′yu Fylm-17	72	3754	10.62	2.83	1.08	
	Average	**72**	**3682**	**10.39**	**2.82**	**1.02**	
LSD_{05}	A	0,93	10,29	0.05	0.11	0.02	
LSD_{05}	B	0,82	5,91	0.04	0.06	0.04	

The correlation analysis between the elements of the crop structure and the seed productivity of sesame plants was carried out. The correlation between the number of pods on one plant and seed yield was 0.311, between the number of seeds on one plant and seed yield was 0.939, the weight of seeds on one plant and seed yield was 0.901, and the weight of 1000 seeds and seed yield was 0.251 (Figure 2).

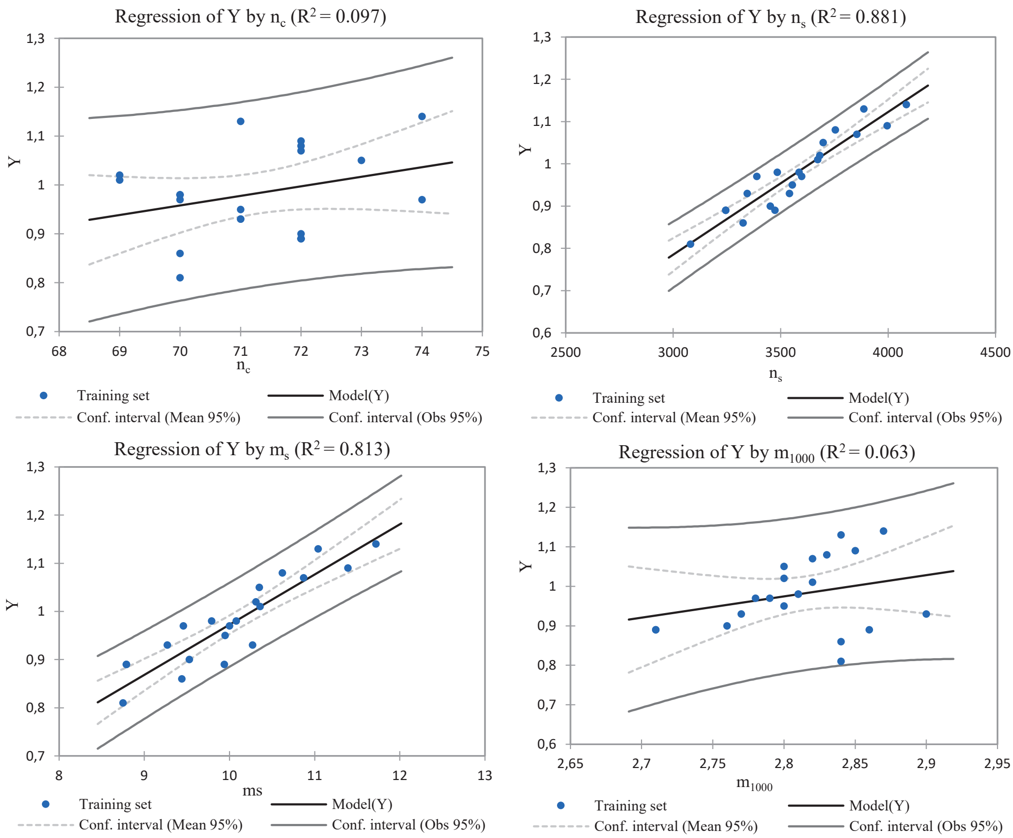

Figure 2. Regression diagrams of the number of pods per 1 plant (n_c) and seed yield (Y) (top left), the number of seeds per 1 plant (ns) and seed yield (Y) (top right), seed weight per 1 plant (m_s) and seed yield (Y) (bottom left), weight of 1000 seeds (m_{1000}) and seed yield (Y) (bottom left)

The advantage of preparations based on adhesives and surface-active substances is: prevention of premature cracking of pods, increase of seed yield and improvement of its quality due to reduction of losses during ripening, which allows collecting uniform ripe seeds. Thus, according to our observations, the best effect was precisely from the use of the drug Agrolip (Figure 3).

Figure 3. The effect of preparations based on adhesives and surface-active substances on the cracking of pods:
1 - Control, 2 - Raps kley, 3 - Agrolip, 4 - N'yu Fylm-17

CONCLUSIONS

The technology of growing sesame should be largely based on the use of potential possibilities of preparations based on adhesives and surface-active substances and desiccants and helps to preserve the seeds in the pod. The best result among desiccants was when processed in the phase of 70-75% browning of pods in Preparation 2 with a seed yield of 1.18 t/ha, which was higher than the control variant by 0.33 t/ha. The best variant when combining preparations based on adhesives and surface-active substances and desiccants turned out to be the variant when applied in the first treatment Agrolip and the second - Desiccant + Agrolip with a seed yield of 1.14 t/ha.

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ECOLOGICAL PLASTICITY AND STABILITY OF WINTER WHEAT VARIETIES IN THE CONDITIONS OF SOUTHERN UKRAINE

Yurii LAVRYNENKO[1], Andrii TYSHCHENKO[1], Halyna BAZALII[1], Vira KONOVALOVA[2], Andrii ZHUPYNA[1], Olena TYSHCHENKO[1], Olena PILIARSKA[1], Tetiana MARCHENKO[1], Kateryna FUNDYRAT[1]

[1]Institute of Climate Smart Agriculture of the National Academy of Agrarian Sciences of Ukraine, 24 Mayatska Doroga Street, sett. Khlybodarske, Odesa District, Odesa Region, 67667, Ukraine
[2]Askanian State Agricultural Research Station of the Institute of Climate Smart Agriculture of the National Academy of Agrarian Sciences of Ukraine, Tavrichanka village, Kakhovka District, Kherson Region, 74862, Ukraine

Corresponding author email: izz.biblio@ukr.net

Abstract

The aim of the research was to study the ecological plasticity and stability of winter wheat varieties under the arid conditions of the southern steppe of Ukraine. The research was conducted during 2015/16–2019/20 at the Institute of Irrigated Agriculture, NAAS, and the Askanian State Agricultural Research Station, Kherson region, Ukraine. The material for the research was 10 varieties of winter wheat which were sown under conditions of optimal (irrigation) and stress (without irrigation) moistening. The response of winter wheat cultivars to growing conditions was analyzed using regression coefficient, homeostatic parameters, general adaptability, variance of specific adaptability, selection value of genotype and others. The minimum yield of varieties varied from 2.02 t ha^{-1} to 3.72 t ha^{-1} and the maximum - from 8.10 to 9.81 t ha^{-1}. The obtained results are a contribution to the study of both theoretical and practical aspects of wheat drought resistance and can be used as elements of selection programs.

Key words*: irrigation, natural moistening, eco gradient, homeostatic, yield.*

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most important crops in maintaining food security which ensures the existence of a large part of the world's population (Franco et al., 2018; Galetto et al., 2017). Scientific predictions suggest that while the world population is rapidly growing, food production will not keep pace with such growth and, given the current dynamics, it is possible that the food problem will cause a deep international crisis. The scientists' estimations show that at the current rate of population growth, the world grain production per capita will decline (Carlson, 2016; Tyshchenko et al., 2020[b]).

At present, the annual gross production of wheat increases by about 0.9%, but it is much slower than the population growth rate and, accordingly, insufficient to meet their needs (Lavrynenko et al., 2019[a], Ray, 2013). Therefore, humanity must find a solution to this problem because the rate of population growth remains too high (Lavrynenko, 2019[b]). Along with the population growth, in recent decades climate change, the so-called global warming, has been observed which leads to significant fluctuations in winter wheat yields (Anderson, 2020; Vozhehova et al., 2021[a]). Therefore, the efforts of breeders should be focused on creating not only high-yielding varieties, but also those that ensure crop stability in different agro-climatic conditions (Vozhehova et al., 2021[b]; Tyshchenko et al., 2020[a]). To date, scientists have studied the agronomic and physiological mechanisms responsible for crop stability (Ojha & Ojha, 2020). Thus, different varieties may show contrasting responses to environmental conditions due to their interaction.

The aim of the research was to study the ecological plasticity and stability of winter wheat varieties in the arid conditions of the southern steppe of Ukraine.

MATERIALS AND METHODS

The response of winter wheat cultivars to different cultivation conditions was studied at the Institute of Irrigated Agriculture, Kherson, Ukraine (46 ° 44'33 "N; 32 ° 42'28" E; 50 m above sea level) (location A) and at Askania State agricultural research station in the village of Tavrichanka, Kherson region (46° 33'12 "N; 33 ° 49'13" E; 39 m above sea level) (location B) during 2015/16-2019/20. The research was conducted under different conditions of soil: under irrigation and without irrigation. Under natural moisturing conditions, the yield strongly depended on the amount of precipitation during the growing season, especially in the critical growing season (April - May). Average temperatures and amount of precipitation for all experimental seasons are shown in Table 1 together with long-term averages (1961-2005). The 2018/19 season was the most favorable as to natural moistening, as the rainfall during the growing season contributed to the replenishment of soil moisture which ensures normal plant growth and development. The 2017/18 and 2019/20 seasons were very dry, especially the critical growing season (April - May) during which air and soil droughts were observed due to insufficient rainfall and high average daily temperatures.

We studied 10 varieties of winter wheat which are usually grown in southern Ukraine and entered in the State Register of Plant Varieties. The varieties were tested on plots of 50 m² in three replicates by randomized replicates (blocks), the sowing rate being 4.5 million viable seeds per hectare. The research was carried out according to the generally accepted methods, the amount of fertilizers and chemical treatments corresponded to the growing conditions and the occurance of diseases and pests. The studied varieties in both areas were sown in the first decade of October and harvested in July. Under irrigation conditions, watering was carried out at the pre-irrigation soil moisture level of 75% of the lowest moisture content (Table 1).

Table 1. Weather conditions during the research (2015-2020)

	1961-2005		2015/2016		2016/2017		2017/2018		2018/2019		2019/2020	
	T (°C)	P (мм)	T (°C)	P (мм)	T (°C)	P (мм)	T (°C)	P (мм)	T (°C)	P (мм)	T (°C)	P (мм)
Askania SARS												
October - December	4.8	98.0	6.0	81.2	3.4	42.0	5.9	75.0	5.5	53.4	7.4	67.9
January	-3.1	30.0	-3.1	59.9	-3.9	14.4	0.7	24.1	-0.3	33.8	1.0	18.3
February	-2.0	29.0	3.9	32.9	-0.9	22.0	0.1	47.0	1.1	10.6	2.2	59.6
March	2.2	26.0	6.1	20.3	6.6	10.2	1.5	35.1	5.5	5.7	7.5	3.5
April	9.6	28.0	12.4	50.5	8.5	81.8	12.9	2.7	10.3	38.9	9.5	7.5
May	15.6	38.0	15.9	95.7	15.5	25.8	19.5	13.0	17.4	72.4	14.9	42.4
June	20.0	46.0	21.5	76.2	21.7	8.0	22.4	23.0	24.5	14.1	22.2	59.3
January - June	7.1	197.0	9.5	335.5	7.9	162.2	9.5	144.9	9.8	175.5	9.6	190.6
October - June	6.0	295.0	7.8	416.7	5.7	204.2	7.7	219.9	7.7	228.9	8.5	258.5
IIA												
October – December	4.8	104.0	6.3	64.9	3.7	99.4	7.5	88.0	5.4	97.0	7.7	74.3
January	-3.0	33.0	-3.6	67.3	-4.7	27.5	-0.3	24.1	-0.6	23.0	0.9	17.3
February	-1.8	31.0	4.0	30.9	-0.8	13.2	-0.2	33.3	1.4	9.8	2.7	56.4
March	2.3	26.0	6.3	19.5	7.0	5.2	1.5	61.0	5.9	7.3	7.6	6.2
April	10.0	33.0	12.6	56.8	9.3	87.9	14.1	1.6	10.5	56.0	9.8	2.8
May	16.0	42.0	16.2	71.7	16.3	25.6	19.5	35.7	18.0	72.8	14.7	29.3
June	19.9	45.0	22.1	43.0	22.0	10.3	22.9	23.1	23.8	92.6	22.7	45.1
January - June	7.2	210.0	9.6	289.2	8.2	169.7	9.6	178.8	9.8	261.5	9.7	157.1
October - June	6.0	314.0	8.0	354.1	6.0	269.1	8.6	266.8	7.6	358.5	8.7	231.4

Source: Data of meteorological station "Askania Nova"

Statistical analysis. The response of winter wheat cultivars to growing conditions was studied using the index of environmental conditions (Ij), regression coefficient (b_i), predictable ecological stability, plasticity of the cultivar at different eco gradients (S_{di}^2) determined by Eberhart S.A., Russell W.A. (Eberhart & Russell, 1966), indicators of stress resistance (Ymin. - Ymax.) and genetic flexibility (Gf) according to the equations by Rosielle A.A., Hamblin J. (Rosielle & Hamblin, 1981), parameters of homeostatic (Hom) and selection value (Sc) according to Hangildin V.V. et al. (Hangiydyn & Lytvynenko, 1981), the adaptability coefficient (AC) by the method of Zhyvotkova L.A. et al.

(Zhyvotkov et al., 1984). General adaptive capacity (GAC), specific adaptive capacity variance (σ^2_{SACV}), relative genotype stability (s_{gi}), genotype selection value (GSV), nonlinearity (l_{gi}) and destabilization compensation (K_{gi}) coefficients were determined according to A. Kilchevsky et al. (Kylchevskyi & Hotyleva, 1985).
A correlation analysis between grain yield and drought resistance indices was performed to determine the best drought resistant varieties and indices. The principal components analysis (PCA) was performed on the basis of observations. Both correlation and PCA were performed using Microsoft ® Excel 2013/ XLSTAT © -Pro (version 2015.6.01.23953, 2015, Addinsoft, Inc., Brooklyn, New York, USA).

RESULTS AND DISCUSSIONS

The obtained experimental data allow to single out the winter wheat varieties according to their maximum productivity. They are *Kokhana* (9.81 t ha^{-1}) and *Mariia* (9.7 t ha^{-1}), the *Koshova* variety being the least productive (3.72 t ha^{-1}).
The stress resistance of the studied winter wheat varieties is reflected by the index of the difference between the minimum and maximum yields (Ymin. - Ymax.), and the smaller this difference, the higher its resistance to stress. According to this indicator, the following winter wheat varieties were singled out: *Rosynka* (-5.08), *Ledia* (-5.20) and *Koshova* (-5.52), but the first two varieties were characterized by lower yields than the average variety (Table 2).

Table 2. Homeostasis, ecological plasticity and adaptability of winter wheat varieties on the basis of grain yield (average for 2016-2020)

Variety, population	Sign	Yield, t ha^{-1}		Adaptability parameters						
		Ymin. - Ymax.	Ymean	Ymin. - Ymax.	Sc	Gf	b_i	S_{di}^2	AC	Hom
Anatoliia	G1	3.24-9.40	6.65	-6.16	2.29	6.32	1.01	0.073	102.4	1.302
Burhunka	G2	2.79-9.20	6.53	-6.41	1.98	6.00	1.06	0.099	100.6	1.207
Konka	G3	2.78-8.68	6.50	-5.90	2.08	5.73	0.96	0.149	100.1	1.299
Kokhana	G4	3.21-9.81	6.65	-6.60	2.17	6.51	1.05	0.177	102.4	1.214
Koshova	G5	3.72-9.24	6.84	-5.52	2.75	6.48	1.00	0.179	105.3	1.536
Mariia	G6	3.19-9.70	6.78	-6.51	2.23	6.45	1.07	0.109	104.4	1.281
Ledia	G7	3.15-8.35	6.21	-5.20	2.34	5.75	0.94	0.100	95.6	1.344
Rosynka	G8	3.02-8.10	5.91	-5.08	2.20	5.56	0.92	0.053	91.1	1.248
Khersons`ka bezosta	G9	2.02-8.43	6.34	-6.41	1.52	5.23	0.96	0.235	97.6	1.136
Askaniis`ka	G10	270-8.70	6.53	-6.00	2.02	5.70	1.03	0.107	100.5	1.287
Average variety			**6.49**	**-5.98**	**2.16**	**5.97**	**1.00**	**0.128**	**100.0**	**1.285**
V, %			4.29	-9.17	14.42	7.49	5.29	43.52	4.27	8.27
$S\dot{x}_{absolute}$			0.09	0.17	0.10	0.14	0.02	0.02	1.35	0.03
$S\dot{x}_{relative}$			1.36	-2.90	4.56	2.37	1.67	13.76	1.35	2.61
$LSD_{0.01}$			0.28	0.55	0.31	0.45	0.05	0.06	4.28	0.11
$LSD_{0.05}$			0.20	0.40	0.23	0.32	0.04	0.04	3.09	0.08

Source: Authors' concept of the experiments

The selection value (Sc) reflects the average yield increase and the ratio between the minimum and maximum yields over the years of research.
The characteristics of the samples with regard to stress are supplemented by the indicator of genetic flexibility (Gf), which shows the average yield of varieties in contrasting (optimal and limiting) conditions. High values of this indicator testify to a high degree of correspondence between the variety genotype and environmental factors. According to this indicator, the varieties of winter wheat such as *Kokhana* (6.51), *Koshova* (6.48), *Mariia* (6.45) and *Anatoliia* (6.32) which form a higher yield under contrasting conditions compared to other varieties have been singled out.
The regression coefficient (b_i) is a criterion (index) for assessing the level of ecological plasticity and indicates the genotype response to changes in environmental conditions, the varieties with $b_i > 1$ are more sensitive to changes in growing conditions. The best varieties of intensive type were *Mariia* (b_i =

1.07) and *Burhunka* (b_i = 1.06). The genotypes with $b_i < 1$ are less responsive to changes in the eco-gradient than the average of the studied varieties and they are important because of their sufficient productivity at a minimum cost. In our research, such varieties include *Rosynka* (0.92) and *Ledia* (0.94). If $b_i = 1$, the genotype is well adapted to different growing conditions and is universal, this is typical of the *Koshova* variety.

On analyzing winter wheat cultivars according to their deviation variance from the S_{di}^2 regression line, the *Rosynka* cultivar with its highest predictable stability of S_{di}^2 equaling 0.053 was selected.

The adaptability coefficient (AC) reflects the ratio of the variety average yield to the average yield of all varieties. High variety adaptability ensures stable yield under different growing conditions, so an important characteristic of the variety is its ability to stably realize the yield potential. The *Koshova* (105.3) and *Mariia* (104.4) varieties were characterized by the highest values.

An indicator of plant resistance to adverse environmental factors is homeostasis (Hom) which characterizes the ability of plants to develop normally under adverse environmental conditions. The *Koshova* variety was characterized by the highest value of homeostasis (1.536).

The greatest values of general adaptability (GAC) were observed in such winter wheat varieties as *Koshova* (0.34) and *Mariia* (0.29), while the *Rosynka* variety was characterized by the lowest value (0.58) (Table 3).

Table 3. Adaptivity parameters of winter wheat varieties based on grain yield (average for 2016-2020)

Variety, population	Sign	Yield, t ha^{-1}		Adaptability parameters						
		Ymin. - Ymax.	Ymean	GAC	$\sigma^2_{(G\times E)gi}$	σ^2_{SACV}	s_{gi}	GSV	K_{gi}	l_{gi}
Anatoliia	G1	3.24-9.40	6.65	0.16	0.03	3.72	29.0	3.40	1.04	0.008
Burhunka	G2	2.79-9.20	6.53	0.04	0.07	4.10	31.0	3.12	1.14	0.016
Konka	G3	2.78-8.68	6.50	0.01	0.11	3.43	28.5	3.38	0.96	0.032
Kokhana	G4	3.21-9.81	6.65	0.15	0.14	4.14	30.6	3.21	1.15	0.033
Koshova	G5	3.72-9.24	6.84	0.34	0.13	3.76	28.4	3.57	1.05	0.035
Mariia	G6	3.19-9.70	6.78	0.29	0.08	4.23	30.3	3.31	1.18	0.020
Ledia	G7	3.15-8.35	6.21	-0.29	0.07	3.27	29.1	3.16	0.91	0.021
Rosynka	G8	3.02-8.10	5.91	-0.58	0.04	3.07	29.6	2.96	0.86	0.012
Khersons\`ka bezosta	G9	2.02-8.43	6.34	-0.16	0.19	3.55	29.7	3.16	0.99	0.053
Askaniis\`ka	G10	270-8.70	6.53	0.03	0.07	3.91	30.3	3.19	1.09	0.017
Average variety			**6.49**	**0.00**	**0.09**	**3.72**	**29.7**	**3.25**	**1.04**	**0.025**
V, %			4.29	-278.19	52.93	10.44	3.01	5.31	10.29	54.65
$S\dot{x}_{absolute}$			0.09	0.09	0.02	0.12	0.28	0.05	0.03	0.01
$S\dot{x}_{relative}$			1.36	-87.97	16.74	3.30	0.95	1.68	3.25	17.28
$LSD_{0.01}$			0.28	0.28	0.05	0.39	0.90	0.17	0.11	0.01
$LSD_{0.05}$			0.20	0.20	0.04	0.28	0.65	0.12	0.08	0.01

Source: Authors' concept of the experiments

The stability of the genotype response as to its productivity is determined by the value of the σ^2_{SACV} parameter. The variance parameter (σ^2_{SACV}) characterizes the specific adaptive ability, that is, under favorable environmental conditions a variety with a high value of this indicator forms a relatively high yield. The following varieties were determined as the most stable: *Rosynka* (σ^2_{SACV} = 3.07), *Ledia* (σ^2_{SACV} = 0.27) and *Konka* (σ^2_{SACV} = 3.43). The *Mariia* variety with the value of σ^2_{SACV} equaling 4.23 is determined as unstable.

The relative stability parameter of the genotype (s_{gi}) is not related to its overall adaptive capacity and is relative. The lowest relative stability values of the genotype were determined in the following varieties: *Koshova* (28.4), *Konka* (28.5) and *Anatoliia* (29.0), which characterizes them as the most stable.

The *Anatoliia* variety was characterized by the lowest value (0.03) of the genotype variance and the environment interaction $\sigma^2_{(G\times E)gi}$, but it was unstable, which testifies to the manifestation of a destabilizing effect. The compensation coefficient varied from 0.86 to 1.18. In such varieties as *Anatoliia, Burhunka, Kokhana, Koshova, Mariia* and *Askaniis'ka* it was more than one, which testifies to the predominance of the destabilizing effect. When selecting stable varieties, preference should be given to varieties with $K_{gi} < 1$.

The genotype selection value (GSV) is used for selecting simultaneously as to general adaptive ability and stability. High genotype selection value (GSV) characterizes such varieties: as *Koshova, Anatoliia and Konka,* their values being 3.57, 3.40 and 3.38, respectively. Varieties of this type are the most valuable and can produce maximum yields even in adverse conditions.

The adaptability coefficient (AC) and general adaptability (GAC) had a high correlation (r = 0.857) with maximum productivity and medium correlation (r = 0.402 and 0.401, respectively) with minimum productivity (Table 4). A number of researchers (Aniskov & Safonova, 2020, Khabibullin et al., 2020, Lozynskyi, 2018) studying the adaptability of different crops believed that these indicators can identify a stable variety. However, in our studies, the highest values of these indicators characterized the varieties of intensive type.

The regression coefficient (b_i) had a high correlation (r = 0.864) with the maximum yield and low correlation (r = 0.196) with the minimum one. Studies by S. A. Eberhart and W. A. Russell presented a gradation: $b_i > 1$ - intensive type varieties, $b_i < 1$ - stable type varieties and $b_i = 1$ - plastic type varieties. Our research and studies by a number of authors (Aseieva & Zenkina, 2019, Buhaiov & Horenskyi, 2017, Ivaniuk et al., 2017, Vozhehova et al., 2022[c]) confirm this regularity.

The specific adaptive capacity variance (σ^2_{SACV}) was characterized by high correlation (r = 0.869) with maximum yield and low correlation (r = 0.144) with minimum yield. A number of authors (Gudzenko, 2019; Ignatiev & Regidin 2019; Lavrynenko, 2019[b]; Lozynskyi, 2018; Vozhehova et al., 2021[b]) believe that the smaller the value of the specific adaptive capacity, the more stable the variety. This is confirmed by our research, but if the value of σ^2_{SACV} variance tends to the maximum, then such varieties should be considered as intensive type.

The selection value of the variety (Sc) and homeostasis (Hom) had low correlation (r = 0.298 and 0.117, respectively) with maximum yield and high correlation (r = 0.972 and 0.781, respectively) with minimum yield. A number of authors (Demydov et al., 2019; Mel'nyk et al., 2020; Postolati et al., 2017) believe that the higher the value, the more stable the variety, which was confirmed by our research.

The relative stability of the genotype (s_{gi}) had a medium negative correlation (r = -0.302) with the minimum yield and the average (r = 0.312) with the maximum yield, i.e. the smaller the value of the relative stability of the variety genotype, the higher its productivity under limiting moisture conditions.

The genotype selection value (GSV) has a medium correlation (r = 0.508-0.509) with minimum and maximum yield.

The compensation coefficient (K_{gi}) had a high correlation (r = 0.871) with the maximum yield, but there was no correlation with the minimum yield (r = 0.148). That is, when selecting varieties of intensive type, preference should be given to varieties with a destabilizing effect ($K_{gi} > 1$), while as for stable varieties the $K_{gi} < 1$ (compensating effect) should be preferred.

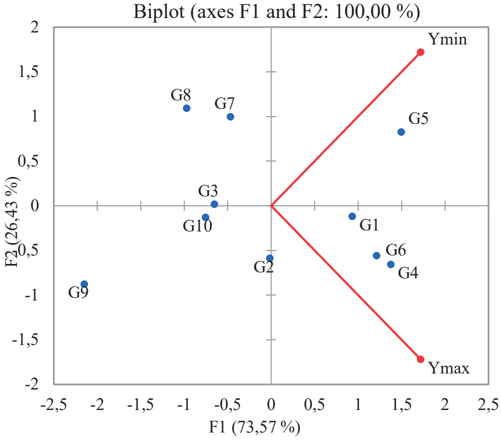
Table 4. Matrix of correlations between maximum and minimum yields of winter wheat varieties and homeostatic, ecological plasticity and adaptability parameters (average for 2016-2020)

	Ymax.	Ymin.	Ymean	Ymin. - Ymax.	Sc	Gf	b_i	S_{di}^2	AC	Hom	GAC	$\sigma^2_{(G\times E)gi}$	σ^2_{SACV}	s_{gi}	GSV	K_{gi}	l_{gi}
Ymax	**1.000**	0.471	0.854	-0.691	0.298	0.898	0.864	0.119	0.857	0.117	0.857	0.064	0.869	0.312	0.509	0.871	-0.067
Ymin	0.471	**1.000**	0.400	0.312	0.972	0.811	0.196	-0.322	0.402	0.781	0.401	-0.365	0.144	-0.302	0.508	0.148	-0.398
Ymean	0.854	0.400	**1.000**	-0.592	0.331	0.766	0.771	0.321	1.000	0.384	1.000	0.223	0.799	0.019	0.804	0.805	0.132
Ymin. - Ymax.	-0.691	0.312	-0.592	**1.000**	0.475	-0.302	-0.770	-0.392	-0.593	0.515	-0.594	-0.369	-0.817	-0.584	-0.131	-0.817	-0.254
Sc	0.298	0.972	0.331	0.475	**1.000**	0.682	0.044	-0.307	0.332	0.895	0.331	-0.356	-0.006	-0.456	0.545	-0.002	-0.359
Gf	0.898	0.811	0.766	-0.302	0.682	**1.000**	0.671	-0.082	0.769	0.467	0.768	-0.139	0.648	0.057	0.590	0.652	-0.243
b_i	0.864	0.196	0.771	-0.770	0.044	0.671	**1.000**	0.029	0.773	-0.068	0.774	-0.009	0.988	0.630	0.257	0.988	-0.159
S_{di}^2	0.119	-0.322	0.321	-0.392	-0.307	-0.082	0.029	**1.000**	0.318	-0.072	0.310	0.988	0.180	-0.103	0.316	0.176	0.973
AC	0.857	0.402	1.000	-0.593	0.332	0.769	0.773	0.318	**1.000**	0.384	1.000	0.221	0.800	0.021	0.803	0.806	0.129
Hom	0.117	0.781	0.384	0.515	0.895	0.467	-0.068	-0.072	0.384	**1.000**	0.381	-0.147	-0.087	-0.647	0.704	-0.081	-0.114
GAC	0.857	0.401	1.000	-0.594	0.331	0.768	0.774	0.310	1.000	0.381	**1.000**	0.213	0.800	0.020	0.804	0.806	0.122
$\sigma^2_{(G\times E)gi}$	0.064	-0.365	0.223	-0.369	-0.356	-0.139	-0.009	0.988	0.221	-0.147	0.213	**1.000**	0.145	-0.037	0.197	0.139	0.985
σ^2_{SACV}	0.869	0.144	0.799	-0.817	-0.006	0.648	0.988	0.180	0.800	-0.087	0.800	0.145	**1.000**	0.616	0.286	1.000	-0.006
s_{gi}	0.312	-0.302	0.019	-0.584	-0.456	0.057	0.630	-0.103	0.021	-0.647	0.020	-0.037	0.616	**1.000**	-0.578	0.607	-0.168
GSV	0.509	0.508	0.804	-0.131	0.545	0.590	0.257	0.316	0.803	0.704	0.804	0.197	0.286	-0.578	**1.000**	0.296	0.200
K_{gi}	0.871	0.148	0.805	-0.817	-0.002	0.652	0.988	0.176	0.806	-0.081	0.806	0.139	1.000	0.607	0.296	**1.000**	-0.010
l_{gi}	-0.067	-0.398	0.132	-0.254	-0.359	-0.243	-0.159	0.973	0.129	-0.114	0.122	0.985	-0.006	-0.168	0.200	-0.010	**1.000**

* - Confidence interval (%): 95

Source: Authors' concept of the experiments

According to the results of GGE biplot analysis, such winter wheat varieties as *Anatoliia* (G1), *Kokhana* (G4), *Koshova* (G5) and *Mariia* (G6), which are between the vectors of yield level, can be distinguished as plastic, i.e. those that form high yields under different growing conditions (Figure 1).



Source: Authors' concept of the experiments

Figure 1. Genotype-environmental interaction of winter wheat varieties and environments (biplot analysis method). The lines show the eigenvectors of the leading factor loads for the environments:
● - yield level; ● - varieties.

The *Ledia* (G7) and *Rosynka* (G8) winter wheat varieties are in quarter IV and are characterized by the smallest decrease in yield under deteriorating conditions, they can be considered the most stable, i.e. those that are tolerant to changes in moisture conditions.

The *Burhunka* winter wheat variety (G2) located on the border of the second and third quarters is characterized by high productivity (9.20 t ha^{-1}) under optimal conditions and average productivity (2.79 t ha^{-1}) under unfavorable ones. This variety can be defined as an intensive type, i.e. one that responds well to improving moisture conditions but is characterized by a sharp decrease in productivity under stressful conditions.

CONCLUSIONS

According to homeostasis, ecological plasticity, parameters of adaptability and biplot-analysis, winter wheat varieties are divided into groups according to different growing conditions:

– the *Ledia* and *Rosynka* varieties are stable (extensive type), i.e. those that respond poorly to changes in moisture conditions and are recommended for natural moisture conditions;

– the *Anatoliia* and *Koshova* varieties are plastic (they form a high yield under different growing conditions) and recommended for cultivation both under irrigation and natural moisture;

– the *Burhunka, Kokhana* and *Mariia* varieties are of intensive type (they form the highest yield under optimal conditions) and are recommended for cultivation under irrigation.

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THE IMPACT OF THE PEA SEED TREATMENTS WITH BIO-FERTILIZERS AND BIO-AGENTS ON THE LEVEL OF PLANT NITROGEN SYMBIOTIC FIXATION

Svitlana LEMISHKO, Mykola KHARYTONOV, Valentina PASHOVA, Yurii TKALICH

Dnipro State Agrarian and Economic University, 25 Serhii Yefremov Street, Dnipro, Ukraine

Corresponding author email: kharytonov.m.m@dsau.dp.ua

Abstract

Main objective was to estimate impact of the pea seed treatments with bacterial fertilizers and bio-agents on the symbiotic nitrogen fixation process in the soil-plant system. Treatments of pea seed with bacterial preparations and bio-agents had different effects chemosynthesis with using nitrification energy derived from reactions involving inorganic chemicals in the rhizosphere of pea. The highest level of soil nitrification energy in the pea rhizosphere was recorded after phosphorus solubilization bacteria (PSB) + Paenibacillus polymyxa (PP) treatment option in the topsoil. The introduction of mineral fertilizers into the soil inhibited the growth of nitrification energy in the pea rhizosphere slightly. The highest pea seeds inoculation effect was observed when phosphorus-mobilizing bacteria (PSB) were combined with Paenibacillus polymyxa (PP). The maximum level of symbiotic nitrogen fixation was noted after pea seed treatments with bio- agent Agat-25 K and PGR Reacom-C.

***Key words**: seed treatments; pea varieties; bio-fertilizers; bio-agents; nitrogen symbiotic fixation.*

INTRODUCTION

Field pea grains contain approximately 21-25% protein including high levels of amino acids, lysine and tryptophan, which are relatively low in cereal grains (Mishra et al., 2010). Crop land use change in Ukraine since 2000 has been mainly in favour of industrial crops (oilseed) and within the cereal area, in favour of corn (Fileccia et al., 2014). Such negative phenomena as irrational exploitation, the lack of scientifically based crop rotation, fertilization and protection systems led to a decrease in the area of pea sowing, yield, and protein content from 22.5-23.5 to 19-22% (Lemishko, 2020). The rhizosphere is the area where plant roots and soil composition interact each with other (Chamkhi et al., 2022). During the process of N conversion formed by microorganisms ammonium salt oxidized to nitrate by nitrification (Xiong et al., 2021). The reaction of peas to the availability of nutrients in the soil is closely related to the activity of nodule bacteria. It was established that this crop needs a small amount of nitrogen in the I-III stages of organogenesis, and in subsequent periods the need for nitrogen is replenished due to nitrogen fixation by its nodule bacteria (Jensen, 1987; Date, 2000). It was established that nitrogen fertilizers should be applied taking into account the level of nitrogen symbiotic fixation is 50-70% of the total pea need.

The implementation of various agricultural measures aimed at improving the conditions of nitrogen nutrition leads to an increase in the protein content of grain (Ahmed et al., 2007; Erman et al., 2009; Geneva et al, 2011). Combination of *Nitragina* inoculant with micronutrient fertilization significantly increased root length density compared to control (Klimek-Kopyra et al., 2018). Significant positive correlations between shoot biomass and nodule biomass or number were observed independently of species affiliation and breeding history (Bourion et al., 2018). Dual inoculation of pea plants increased growth, mycorrhizal colonization rate, nodulation parameters, N_2 fixation activity (Stancheva et al., 2006). The maximum number of nodules per plant were recorded where composite culture of Rhisobium+PSB (Tyagi et al., 2003). Meantime, the application of 10 kg N/ha reduced the nodules number mainly because of medium status of organic matter content of soil and low requirement of chemical nitrogen of pea. Pea seeds inoculation led also to increase the number of pods plant^{-1}

by 2 times (Mekonnen and Assefa, 2019). The co-inoculation of Rhizobium + PSB at 40 mg P kg^{-1} soil significantly increased shoot and root length, number of flowers, pod, and nodules per plant, root and shoot dry weight, 100 grain weight and number of grains per pod (Abid et al., 2016). The combined application of *Glomus fasciculatum* and *Rhizobium* also remarkably increased the nitrogen and phosphorus contents of pigeon pea (Bhattacharjee and Sharma, 2012). The results suggest that dual inoculation of *Glomus fasciculatum* and *Rhizobium* revealed synergistic effect to enhance the chlorophyll, nitrogen and phosphorus contents of pigeon pea. The increasing need to stipulate seeds germination, to control plant pathogens and insects in organic agriculture promotes the search for safe and effective compounds from natural sources, especially plant-derived compounds (Chang et al., 2022). Effect of two plant growth stimulators: bactozol (bacterial origin) and D1 (synthetic analog of phytohormones) on metabolism of pea rhizobia (*Rhizobium leguminosarum* bv. *viciae* 2636) and efficiency of their symbiosis with pea plants have been studied (Kosenko et al., 2001). Treatment of pea plants by bactozol (0.1%) increased the efficiency of their symbiosis with pea rhizobia, evoking the growth of the overground and root mass of the plants, quantity of nodules and their nitrogenase activity.

Main objective of this case study was to estimate impact of the pea seeds treatment with bacterial fertilizers and bio-agents on the symbiotic nitrogen fixation process in the soil-plant system.

MATERIALS AND METHODS

The series of field experiments with bio-fertilizers, bio-stimulants and bio-fungicides were conducted in Verkhnedniprovsky district of Dnipropetrovsk province situated in the northern part of the steppe zone of Ukraine from 2006-2008 and 2015-2017 years (Lemishko, 2020). This research was carried out in a six-field chain of grain-row crop rotation. After harvesting the predecessor of peas (winter wheat), disking was carried out to a depth of 10-12 cm, followed by shelf plowing to a depth of 20-22 cm. Early spring tillage consisted of leveling the soil, pre-sowing cultivation, and application of mineral fertilizers with a dose of $N_{20}P_{40}$ (ammonium nitrate, granular superphosphate). Sowing was carried out with a seeder with 15 cm row spacing at the optimal time when the soil was physically ripe. The object of research was the two pea varieties Kharkiv yantar and Kharkiv etalon.The distribution of precipitation by month is extremely uneven. The average annual rainfall is 455 mm. The content of humus in the top of black soil is 4.5%. The scheme of the field experiment included control and five seeds bacterial inoculation separately, dual and triple: control; *Rhizobium leguminosarum* (R) at the dose of 200 g/ton of seeds; *Bacillus* megaterium phosphorus solubilization bacteria (PSB) - 455 ml/ton of seeds; *Paenibacillus polymyxa* (PP) - 600ml/ton of seeds; R+PSB; R+PP and R+PSB+PP. The control trial included seeds water treatment accepted in the field experiment. Pea seeds before sowing were treated also with biological preparations of plant growth, fungicidal and insecticidal action. Emistym S is the complex of growth substances in a water-alcohol solution, which includes phytohormones of auxin, cytokinin, and gibberellin nature, trace elements, carbohydrates, and fatty acids. These substances complex is made from derivatives of the metabolism of epiphyte fungi, obtained from the roots of medicinal plants. Agate-25K is the multifunctional bio-agent of fungicidal action with plant growth action. The active substance is inactivated bacteria (titre 5-8x1010 cells / ml before inactivation) *Pseudomonas aureofaciens* N 16 and products of their metabolism. Mikosan-N fungicidal activity based on extracts of mushroom glucans (Melo et al., 2020). Reacom-C-beans action is based on chelates of essential trace elements (Cu, Zn, Mo, Mn, Co, B) in a biologically active and plant-available form. The composition of the PGR Vympel - K includes polyatomic alcohols - up to 300 g/l, humic acids - 30 g/l and carboxylic acids of natural origin - 3 g/l (Katelevsky et al., 2020). It is a typical plant growth regulator with adhesive, adaptogenic and cryoprotective effect. Ganol is extract of wormwood (*Artemisia* spp. L.) to use it as product against some seed-borne pathogens in substitution for the chemical pesticides

(Dadasoglu et al., 2015). Its antiseptic action allows for the reduction of damage to plants by phytopathogenic organisms and limits the population of pests at all stages of development. The nitrogen concentration in plant samples was estimated using Kjeldahl method. The coefficient of nitrogen fixation or a number that shows the amount, the ratio of nitrogen from its total removal with the crop, which falls on the fate of fixation by nodule bacteria by peas was determined in the variety without fertilizers (Scherbakov and Rudai, 1983). Data base received in field experiments accomplished were processed by statistical methods using the software package StatGraphics Plus5 with all tests of significance being made at a type 1 error rate of 5%.

RESULTS AND DISCUSSIONS

Inoculation of pea seeds with bacterial fertilizers had different effects chemosynthesis using nitrification energy derived from reactions involving inorganic chemicals in the rhizosphere of pea variety Yantar (Figures 1 and 2).

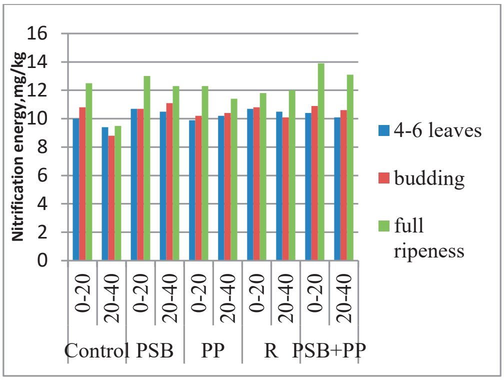

Figure 1. Nitrification energy of soil after bio-fertilizers application (without mineral fertilizers)

The increase in the rate of soil nitrification in the trial without the application of mineral fertilizers continued until the stage of full ripeness. The highest level of soil nitrification energy in the pea rhizosphere was recorded in the PSB+PP treatment option in the topsoil (0-20 cm).

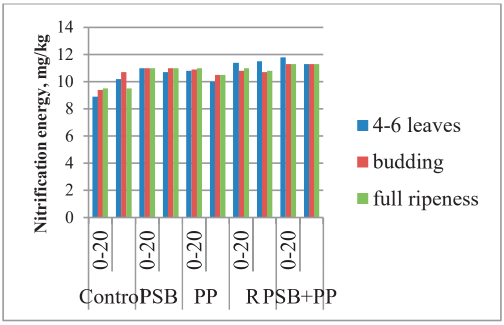

Figure 2. Nitrification energy of soil after bio-fertilizers application (with mineral fertilizers)

Growth of soil nitrification rates in the trial with the initial application of mineral fertilizers ended in the first two stages (4-6 leaves and budding). The highest level of soil nitrification energy in the pea rhizosphere was recorded also in the PSB+PP treatment option in the topsoil. However, the introduction of mineral fertilizers into the soil inhibited the growth of nitrification energy in the pea rhizosphere slightly. It is well known that one of the sources of replenishment of nitrogen reserves in the soil is the biological fixation of atmospheric nitrogen by nodule bacteria, which develop better in soils with a neutral reaction of the environment with a sufficient supply of humus and mobile forms of phosphorus and potassium(Caldwell, 2005). Knowing the dynamics of nitrogenase activity in the root zone of plants grown on different fertilization backgrounds (from deficit to excess nitrogen in the soil) the physiologically justified from crop standpoint doses of mineral nitrogen that will not reduce the nitrogen fixation activity can be determined (Ladha et al., 1986). Meantime, the application of 10 kg N/ha reduced the nodules number mainly because of medium status of organic matter content of soil and low requirement of chemical nitrogen of pea (Tyagi et al., 2003). Determining the dimensions of biological nitrogen fixation is of great importance in solving the problem of increasing soil fertility and agricultural productivity in general. Therefore, biological nitrogen fixation makes corrections when studying the cycle and balance of nitrogen in pea crops. The amount of nitrogen fixation depends on the type of leguminous crop (Posypanov and Knyazeva, 1975). Alfalfa with high hay yields fixes up to

150 kg/ha of nitrogen in the soil, and peas - up to 40-100 kg/ha, depending on the level of the harvest. In favorable years, the coefficient of nitrogen fixation is 0.65 (Scherbakov and Rudai, 1983). The coefficient decreased to 0.59 when phosphoric fertilizers (P_{60}) were applied, and to 0.53 on the background of $P_{60}K_{60}$. The coefficient of nitrogen fixation with rate $N_{60}P_{60}K_{60}$, was minimal and amounted to 0.34. The coefficient of nitrogen fixation by pea nodule bacteria in our experiments was 0.5. The use of phosphorus-mobilizing bacteria for the inoculation of Kharkiv Yantar seeds on a background without mineral fertilizers contributed to an increase in nitrogen fixation on 32% (Table 1). The growth of nitrogen fixation in the dual treatment R+PP was 11.4 and 13.2 kg/ha, or 22 and 25%, respectively. The highest nitrogen fixation was observed when phosphorus-mobilizing bacteria were combined with *Paenibacillus polymyxa* (17.1 kg/ha and 33.0%). Phosphorus-mobilizing bacteria in their combination with *Paenibacillus polymyxa* acted best under the conditions of application of mineral fertilizers. The increase in nitrogen fixation, compared to the control, was at the level of 19.0 and 19.3 kg/ha, or 31%. The overall dimensions of nitrogen fixation were slightly lower in the Kharkiv etalon variety on both growing backgrounds than in the Kharkiv yantar variety. However, the trend of the effect of seed inoculants remained. This result confirms earlier data that the *Paenibacillus polymyxa* can be efficiently used together with phosphorus amendment (Lal and Tabacchioni, 2009).

The Yantar variety pea seeds treatment with bio-agents at the background without mineral fertilizer application contributed to an increase in the nitrates content in the soil rhyzosphere compared to treating seeds with control in the stage of 4-6 leaves (Figure 3). The increase in nitrate content was 19% after seeds treatment with Agat-25K, Emistym-C - 10%, and Reacom - 8% in the trial without mineral fertilizers. Vitavax 200 FF (5%) and Vympel (6%) action was less effective. The above dependencies were also observed in the next stages (budding and full maturity). The nitrogen content of nitrates in the background with the use of mineral fertilizers was 8-20% higher than without fertilizers (Figure 4). At the same time, the seeds treatment action with bio-agents remained the same as on the background without the application of mineral fertilizers. The nitrogen content of nitrates in the background with the use of mineral fertilizers was 8-20% higher than without fertilizers. At the same time, the action of incrustation with biological preparations remained the same as on the background without the application of mineral fertilizers.The effect of seed treatment with PGR and bio-agents was manifested at the determined energy of nitrification after seven days of composting.

The maximum level of symbiotic nitrogen fixation was noted after seeds treatment with bio-agent Agat-25 K and PGR Reacom-C (Table 3). The dimensions of symbiotic fixation of the Kharkiv yantar variety after seeds treatment with Reacom-C and Agat-25 K on the background without the use of fertilizers were 62.6 and 64.5 kg/ha, or 22 and 26% more than in the control (treatment with water). The introduction of mineral fertilizer in a minimum dose of $N_{20}P_{40}$ provided better conditions for the formation of nodules and increased nitrogen accumulation with slightly lower rates of symbiotic nitrogen fixation (13.6 and 24.6%). The dimensions of nitrogen fixation of Kharkiv etalon peas were somewhat lower than those of Kharkiv yantar in all trials of the field experiment. Indicators of nitrogen fixation for the Kharkiv etalon variety in the control without mineral fertilizers were 49.4 kg/ha, in the background with mineral fertilizers - 54.7 kg/ha.

The effect and ratio of indicators for the bio-stimulators and bio-agents remained the same as for the Kharkiv yantar variety. The bio-agent Agat-25 K and PGR Reacom - C beans were most effective, under the influence of which the size of nitrogen fixation on the background without fertilizers increased compared to the control by 23 and 21%, and on the background of mineral fertilizers - by 27 and 34%. A positive effect after processing pea leaves on growth indicators, yield and the number of nodules with biological preparations with a growth-regulating effect was recorded in experiments with garden pea (*Pisum sativum* var Hortense) cv. Pusa Pragati (Syngh et al., 2018).

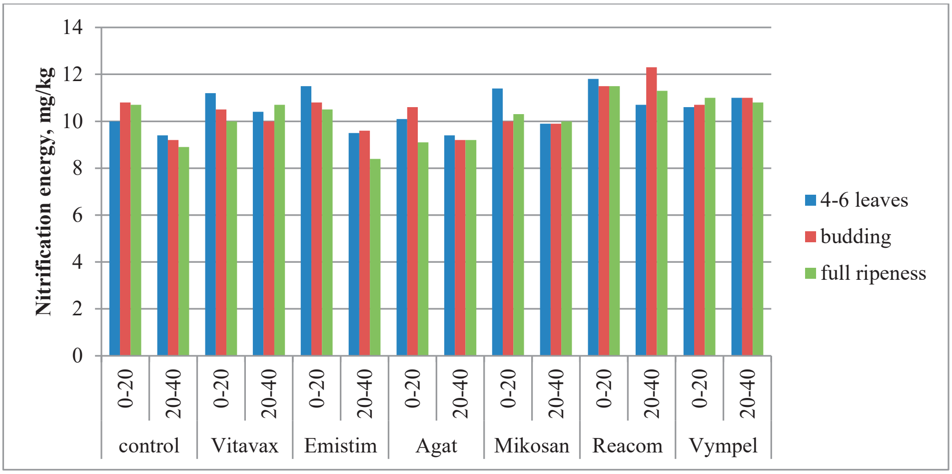

Figure 3. Nitrification energy of soil after bio-stiulators and bio-agents application

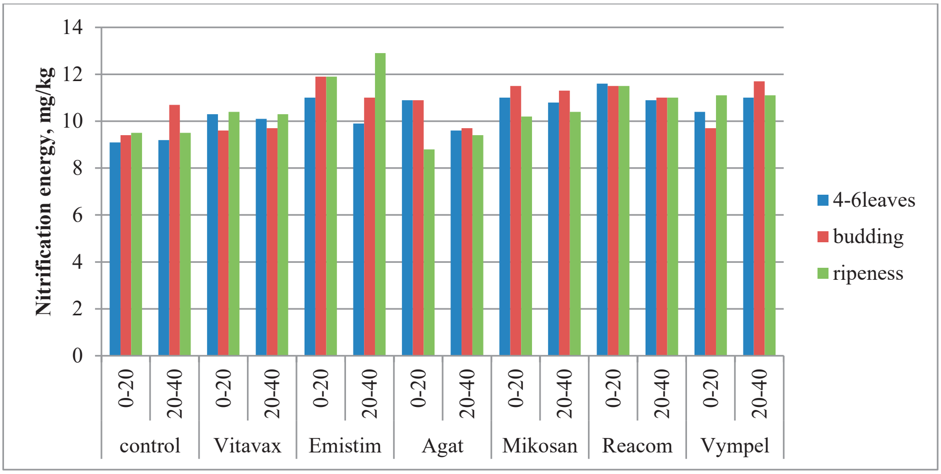

Figure 4. Nitrification energy of soil after bio-stiulators and bio-agents application
(with mineral fertilizers)

Table 1. The effect of inoculation of pea seeds with bacterial fertilizers under different feeding conditions on symbiotic nitrogen fixation, kg/ha

Treatment (factor B)	Kharkivsky yantar (factor A)		Kharkivsky etalon (factor A)	
	without fertilizer (factor C)	$N_{20}P_{40}$ (factor C)	without fertilizer (factor C)	$N_{20}P_{40}$ (factor C)
Control	52.0	61.6	51.0	58.8
PSB	68.5	80.6	65.0	69.2
PP	63.4	75.7	62.4	71.1
Rhizobium leguminosarum (R)	65.2	77.9	64.1	73.7
PSB + PP	69.1	80.9	63.5	71.3
PSB + R	66.1	78.1	61.6	70.5
PP + R	63.4	73.8	60,8	71.6
PSB + PP + R	64,0	73.2	62.8	70.5
LSD $_{0,95}$ kg/ha				
factor A	4.10			
factor B	4.41			
factor C	5.70			
combination of ABC factors	6.01			

Table 2. Symbiotic nitrogen fixation depending on the treatment of pea seeds with bio-agents, kg/ha

Tretament (Factor B)	Kharkiv yantar (factor A)		Kharkiv etalon (factor A)	
	Without fertilizers (factor C)	$N_{20}P_{40}$ (factor C)	Without fertilizers (factor C)	$N_{20}P_{40}$ (factor C)
Water (control)	51.4	62.3	49.4	54.7
Vitavaks-200	58.9	65.7	57.3	63.8
Emistim-C	60.0	70.5	59.2	66.3
Agat–25 K	64.5	70.8	60.4	69.9
Mikosan-N	60.0	69.9	56.0	66.0
Reacom-C	62.6	77.6	59.6	73.0
Vympel	57.6	64.5	55.6	62.3
Ganol	59.2	67.2	56.6	64.0
LSD $_{0,95,}$ kg/ha				
for factor A		3.30		
for factor B		2.90		
for factor C		5.51		
for ABC		6.62		

The results of determining the protein yield after seeds inoculation of both varieties with biological fertilizers (without and with the use of mineral fertilizers) are shown in Figures 5 and 6.

Application of mineral fertilizer with a small dose of $N_{20}P_{40}$ provided the highest yield of protein after seed treatment with *Rhizobium*.

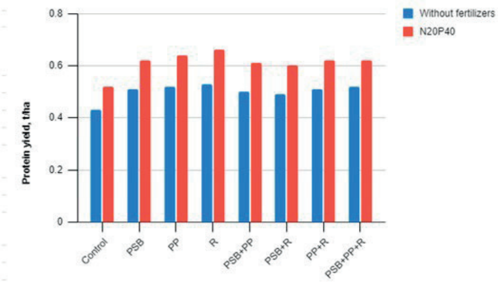

Figure 5. Protein yield of the Kharkiv yantar variety under the influence of seed inoculation with bacterial fertilizers

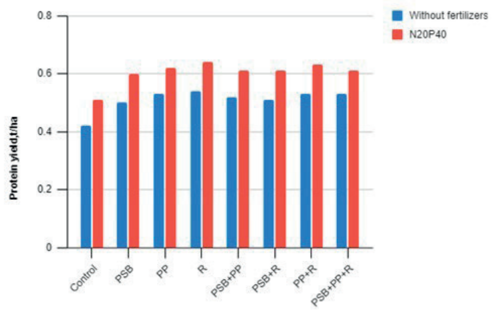

Figure 6. Protein yield of the Kharkiv etalon variety under the influence of seed inoculation with bacterial fertilizers

The results of determining the protein yield after seeds treatments with bio-stimulators and bio-agents of both varieties (without and with the use of mineral fertilizers) are shown in Figures 7 and 8.

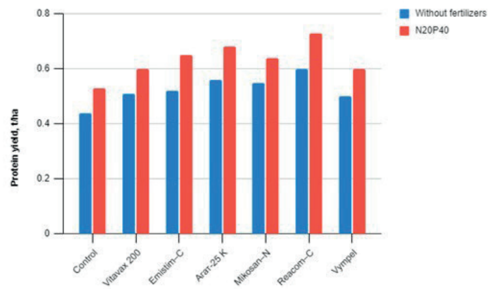

Figure 7. Protein yield of the Kharkiv yantar variety under the influence of seed treatment with bio-stimulators and bio-agents

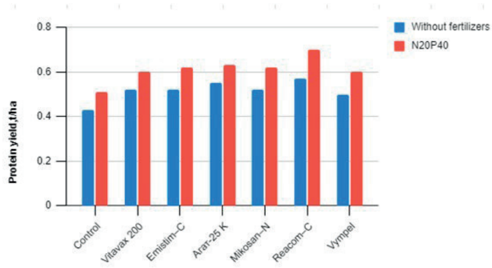

Figure 8. Protein yield of the Kharkiv etalon variety under the influence of seed treatment with bio-stimulators and bio-agents

The highest protein yield was obtained after seeds treatment with Agat-25 K and Reacom-C when $N_{20}P_{40}$ fertilizer was applied.

CONCLUSIONS

Inoculation of pea seeds with bacterial preparations had different effects chemosynthesis with using nitrification energy derived from reactions involving inorganic chemicals in the rhizosphere of pea variety Yantar. The increase in the rate of soil nitrification in the trial without the application of mineral fertilizers continued until the stage of full maturity. The highest level of soil nitrification energy in the pea rhizosphere was recorded at the phosphorus solubilization bacteria (PSB+PP) and *Paenibacillus polymyxa* (PP) treatment option in the topsoil (0-20 cm). The increase in soil nitrification rates in the trial with the initial application of mineral fertilizers ended in the first two stages (4-6 leaves and budding). The increase in nitrogen fixation, compared to the control, was at the level of 19.0 and 19.3 kg/ha, or 31%. The overall dimensions of nitrogen fixation were slightly lower in the Kharkiv etalon variety on both backgrounds than in the Kharkiv Yantar variety. Meanwhile, the trend of action of pea seed inoculants was maintained. Higher costs of using nitrogen, phosphorus and potassium from the soil for the formation of 1 ton of grain were observed with the use of phosphorus solubilization bacteria. Therefore, the pea symbiotic fixation can be improved by using bacterial fertilizers. This approach is much cheaper and easily introduced into the technological cycle of growing this grain crop. It is most appropriate to use for inoculation *Rhizobium leguminosarum* and its combination with PSB+PP. Pea seed treatments with bio-agents Reacom and Agat 25 were the most effective for symbiotic nitrogen fixation.

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RESEARCH ON THE INFLUENCE OF DIFFERENT DOSES OF NITROGEN AND PHOSPHORUS ON YIELD AND QUALITY INDICES ON CORN SEEDS, UNDER PEDOCLIMATIC CONDITIONS AT A.R.D.S. SECUIENI

Alexandra LEONTE[1], Simona Florina ISTICIOAIA[1], Paula PINTILIE[1], Adina Cătălina DRUȚU[2], Andreea ENEA[1], Sabina EȘANU[1]

[1]Agricultural Research and Development Station Secuieni, 371 Main Street, Commune Secuieni, Neamt County, Romania
[2]"Ion Ionescu de la Brad" Technological High School Horia - Neamt, 115 Alexandru cel Bun Street, Commune Horia, Neamt County, Romania

Corresponding author email: andreeapintilie62@gmail.com

Abstract

The experience was in the experimental field of the A.R.D.S. Secuieni, and it was a bifactorial experience, of the 5 x 5 type, in five repetitions so that A factor was represented by different doses of phosphorus (a1 - P_0; a2 - P_{40}; a3 - P_{80}; a4 - P_{120}; a5 - P_{160} kg P_2O_5/ha), and B factor was represented by different doses of nitrogen (b1 - N_0; b2 - N_{40}; b3 - N_{80}; b4 - N_{120}; b5 - N_{160} ,kg N/ha). On average, during the ten years of testing, the productions varied within quite wide limits, from 6252 kg·ha^{-1}, in the variant in which no dose of fertilizer was applied (control - N_0P_0), and the maximum was recorded in the variant in which the dose of fertilizer administered was $N_{160}P_{160}$ active substance, whose production was 9319 kg·ha^{-1}. As for the protein content, it was different from one year to another, the minimum was obtained in the N_0P_{40} variant, in 2019, being 8.1%, and the maximum was obtained in the variant for which the applied dose of fertilizer was $N_{160}P_{120}$, with a protein content of 13.8%.

Key words: *corn, fertiliser, nitrogen, phosphorus, yield.*

INTRODUCTION

Over the last two decades of the 20th century and the first decade of the 21st century, the average corn yield in the world has increased by 70%. This increase has been the result of constant progress in the breeding and development of increasingly fertile hybrids, application of different types and forms of fertilizers, but also the development of agricultural machines that are used to perform the necessary technological operations (Starcevic et al., 2006; Egli, 2008; Assefa et al., 2017).
The current problems in agriculture require the expansion of research in the field of field crop technologies, and obtaining productions under conditions of economic efficiency and protection of the environment is a permanent concern (Lazin, 2014).
Maize (*Zea mays* L.), a plant with great economic value, occupies a large share in the food security of the population, this being a staple food, all over the globe, both in human and animal food. In addition to food and feed, it can also be used in complementary fields: biofuel and as a source of basic raw materials for the food industry, for example, starch, oil, protein, alcoholic beverages, food sweeteners, cosmetics (Ram & Jabeen, 2016).
The fertilization is an important part of the cultivation technologies. The results obtained by the agricultural research and practice have highlighted the influence of this technological link on the qualitative, and quantitative, but even on the economical side of resulted crops (Lupu et al., 2014).
It is well known that nitrogen significantly influences the growth and development of plants. Knowledge of the physiological mechanisms that contribute to the absorption and use of nitrogen are particularly important to increase the efficiency of the use of this chemical element (Agapie et al., 2021).
When nitrogen availability is low in the soil, the plant preferentially supports the growth of nodules and the activity of fixing nitrogen, so when the level of nitrates in the soil is high, the plant stimulates the lateral growth of the roots to absorb the nitrates more efficiently (Saito et al., 2014).

Phosphorus and potassium, applied unilaterally, does not influence the quality of crops, they are used only as a substrate for nitrogen. The best results are obtained by the combined application of the three macro elements, both in terms of productivity and grain quality (Agapie et al., 2018).
Previous studies have also shown changes in root growth with different P fertilizer placements, affecting crop response under drought stress conditions (Hansel et al., 2017a; Hansel et al., 2017b).
Commercial fertilizers containing nitrogen, together with manure, have a large impact on maize production (Xia et al., 2021).
Croitoru (2012) considers that by increasing the doses of fertilizers progressively, increases the yield of corn grains per unit surface and the protein content of the grain, ultimately resulting in an increase in the amount of protein produced per hectare.
The quality and level of production are the result of the interaction between the supply of soil nutrition and the variation of climatic conditions (Pintilie & Sin, 1974; Frye & Thomas, 1991).
In the modern agriculture, the importance of using chemical fertilizers is undeniable. In the structure of chemical fertilizers, those with nitrogen occupy the main place due to their contribution in determining the yield increase, as well as due to the weight with which they participate in the applied fertilization formulas (Mihăilă et al., 1996; Dumitraşcu et al., 2003; Petcu et al., 2003).

MATERIALS AND METHODS

The experience was placed in 2012-2022, at the Agricultural Research and Development Station Secuieni (A.R.D.S. Secuieni) on a typical cambic phaeoziom (chernozem) soil, with medium texture, characterized as being well supplied with phosphorus (P_2O_5 - 39 mg/kg) and mobile potassium (K_2O - 161 mg/kg), moderately supplied with nitrogen, the soil nitrogen index being 2.1, weakly acidic, with pH values (in aqueous suspension) of 6.29 and poorly fertile, with a humus content of 2.3% (Leonte et al., 2021). The experience was placed in a three year rotation: wheat - corn - beans, in a field with no irrigation conditions.
The experience it was set up according to the method of subdivided type, in which the large plot is the dose of phosphorus (0, 40, 80, 120 and 160 kg P_2O_5/ha), and the small one, the dose of nitrogen (0, 40, 80, 120 and 160 kg N/ha). During the experiment, the fertilization was done with ammonium nitrate and superphosphate, and the amount of fertilizer used corresponded to the dose of phosphorus and nitrogen.
In the field, in this case, the cultivation technology specific to the conditions in Central of Moldavia was used, and the data obtained was interpreted statistically according to the method of variance analysis (Ceapoiu, 1968; Jităreanu, 1999).
The analysis of the temperatures and precipitations evolution in the Secuieni area during the vegetation period of corn shows that is becoming increasingly hot and drier, and meteorological extremes can cause very large losses in agriculture.
From the analysis of the average monthly temperature, it resulted that there is an increasing trend compared to the multiannual average, with the deviations recorded in the ten years reaching values from 0.3°C (2013/2014) to 2.2°C (2014/2015) (Table 1).
The temperatures during the corn vegetation season, in the ten years of experimentation, compared to the multiannual average of the last 60 years, five years were normal, four years were warm, and one year was warmer (Table 1).

Table 1. Registered temperatures at A.R.D.S. Secuieni during corn vegetation period

Year	May	Jun.	Jul.	Aug.	Sept.	Dev.
2012/2013	17.7	19.9	20.5	20.4	14.2	0.6
2013/2014	15.5	18.2	20.8	20.6	15.8	0.3
2014/2015	16.6	20.1	22.8	22.4	18.4	2.2
2015/2016	14.9	20.3	21.7	20.6	17.3	1.1
2016/2017	15.8	20.3	20.4	21.2	16.3	0.9
2017/2018	17.8	20	20.3	21.5	16.3	1.3
2018/2019	15.3	21.3	20.1	21.2	16.2	0.9
2019/2020	13.9	20	20.9	22.2	18	1.1
2020/2021	14.7	19.2	22.2	20.5	14.4	0.3
2021/2022	16.3	20.7	22.2	22.7	14.7	1.4
Monthly average	15.9	20.0	21.2	21.3	16.2	1.0
Multiannual average 60 years	15.4	18.9	20.4	19.7	15.1	
Deviations	0.4	1.1	0.8	1.6	1.1	

Under the aspect of precipitation during the entire vegetation period of the corn crop, the deviations from the multiannual average were varied, their distribution was uneven, especialy on the growth and development phenophases of the plant.

The smallest deviations compared to the multiannual average of the same period were 14.5 mm (2018), and the largest deviations were 203.9 mm (2015) (Table 2).

Table 2. Registered precipitation at A.R.D.S. Secuieni during corn vegetation period

Year	May	Jun.	Jul.	Aug.	Sept.	Dev.
2012/2013	51.4	146	76.4	42	42.6	26.5
2013/2014	96.2	66.6	91.1	37	9.2	-31.8
2014/2015	5.6	34	51	12.6	24.8	-203.9
2015/2016	120	161	4	32	48.6	33.9
2016/2017	59.4	49.4	72.2	23	55.2	-72.7
2017/2018	23.4	140	138	4	11.8	-14.5
2018/2019	95	55.8	46.6	20.4	64.8	-49.3
2019/2020	69.6	72.6	39	51.2	60.4	-39.1
2020/2021	31.4	79.4	51.6	76.8	9.2	-83.5
2021/2022	20.8	56.6	35.2	15.2	31	-173.1
Monthly average	57.3	86.2	60.5	31.4	35.8	-60.8
Multiannual average 60 years	64.3	84.7	80.6	58	44.3	
Deviations	-7	1.5	-20	-26.6	-8.5	

RESULTS AND DISCUSSIONS

Climatic conditions during the growing season are the main factor that determines the crop yield, especially in areas where the only source of water is from rainfall and groundwater and even the extreme temperatures can significantly reduce crop yield.

The average yields recorded after the application of different doses of phosphorus (40, 60, 80, 120, 160 kg P_2O_5/ha) indicate that the corn reacts well to this element compared to the control variant (P_0), and the yield differences obtained in the four fertilized variants have statistically ensured and interpreted as highly significant (Table 3).

Table 3. The influence of phosphorus doses on corn yield

Doses P	Yield average kg·ha^{-1}	Relative yield %	Diference	Signifiance
P_0	7391	100	-	Mt.
P_{40}	7723	104	332	***
P_{80}	8089	109	698	***
P_{120}	8338	113	947	***
P_{160}	8270	112	879	***

Phosphorus fertilizers brought increases in yields of 4 to 13% representing 332 to 947 kg·ha^{-1}.

The fertilization of the corn crop with different doses of nitrogen shows an increase in yield from one dose to another, compared to the unfertilized variant (control), but the highest yields were obtained in the variants where the applied doses were 120% (1695 kg·ha^{-1}) and 160% (1979 kg ha^{-1}) active substance, yields that were statistically ensured and interpreted as being very significant.

The increases of yield obtained by applying nitrogen fertilizers were higher than those achieved by applying phosphorus fertilizers and represented 12-29% of their value (Table 4).

Table 4. The influence of nitrogen doses on corn yield

Doses N	Yield average kg·ha^{-1}	Relative yield, %	Dif.	Semnif.
N_0	6816	100	0	Mt.
N_{40}	7631	112	815	***
N_{80}	8055	118	1239	***
N_{120}	8511	125	1695	***
N_{160}	8795	129	1979	***

During 2012-2022, the yields obtained in the corn crop recorded variations due to the combination of fertilizers used, the size of the dose administered but also due to the weather conditions.

Thus, in the ten years of testing, the yields achieved at the unfertilized variant (N_0P_0), varied from 2503 to 8486 kg·ha^{-1}, the average of the period being 6252 kg·ha^{-1} (Table 5).

Compared to this, the increases in yield achieved by applying fertilizers ranged between 10% to 49%, representing 648 to 3097 kg ha^{-1}.

It was noted that nitrogen fertilizers brought higher yield increases the higher the phosphorus agrofond was applied.

Thus, the nitrogen fertilizers in a dose of N_{40} - N_{160} on the P_0 agrofond, achieved yield increases of 12 to 19%, representing 731 to 1213 kg·ha^{-1}; on the P_{40} agrofond, increases of 10 to 27%, representing 648 to 1707 kg·ha^{-1}; on the P_{80} agrofond increases of 13 to 38% representing 830-2380 kg·ha^{-1}; on the agrofond of P_{120}, increases of 21 to 43% representing 1315-2398 kg·ha^{-1} and on the agrofond of P_{160}, increases of 24 to 49% representing 1470 to 3067 kg·ha^{-1} (Table 5).

Table 5. The influence of phosphorus and nitrogen fertilizers on corn yield, in the period 2012-2022

A x B	Variation limits of yield kg·ha^{-1}	Yield average kg·ha^{-1}	Rel. yield %	Dif.	Sem.
$N_0 P_0$	2503-8486	6252	100	-	-
$N_{40} P_0$	4123-9638	6983	112	731	***
$N_{80} P_0$	3767-10072	7358	118	1106	***
$N_{120} P_0$	2814-10261	7456	119	1204	***
$N_{160} P_0$	2310-10130	7465	119	1213	***
$N_0 P_{40}$	2800-10109	6900	110	648	***
$N_{40} P_{40}$	3237-10647	7405	118	1153	***
$N_{80} P_{40}$	2414-11687	7636	122	1384	***
$N_{120} P_{40}$	2146-12200	7929	127	1677	***
$N_{160} P_{40}$	1848-11486	7959	127	1707	***
$N_0 P_{80}$	2496-10874	7082	113	830	***
$N_{40} P_{80}$	2880-12687	7933	127	1681	***
$N_{80} P_{80}$	3196-12450	8183	131	1931	***
$N_{120} P_{80}$	2656-13494	8632	138	2380	***
$N_{160} P_{80}$	1598-12501	8382	134	2130	***
$N_0 P_{120}$	2311-11842	7567	121	1315	***
$N_{40} P_{120}$	3200-12102	8239	132	1987	***
$N_{80} P_{120}$	2168-13517	8542	137	2290	***
$N_{120} P_{120}$	2040-13539	8950	143	2698	***
$N_{160} P_{120}$	1716-13347	8853	142	2601	***
$N_0 P_{160}$	2449-12216	7722	124	1470	***
$N_{40} P_{160}$	3237-12216	8390	134	2138	***
$N_{80} P_{160}$	2880-13440	8788	141	2536	***
$N_{120} P_{160}$	1763-13815	9125	146	2873	***
$N_{160} P_{160}$	2781-14065	9319	149	3067	***

Correlating the doses of phosphorus applied, with the average yields obtained in the ten years of testing, it is observed that there is a direct link between them, the correlation coefficient being statistically ensured and interpreted as distinctly significant (Figure 1).

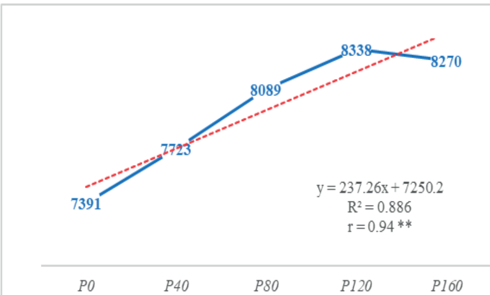

Figure 1. Correlation between yield and phosphorus doses

Regarding the correlation between the applied nitrogen doses and the yield level, which is of the form y = 483.66x + 6510.6, a direct, very close correlation is shown between the two elements with a very significant correlation coefficient (Figure 2).

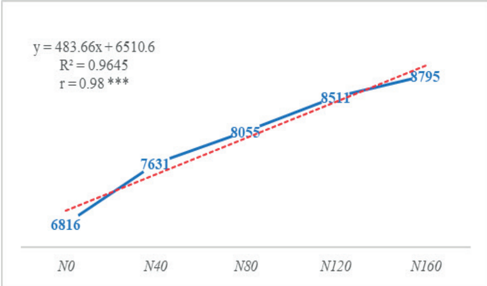

Figure 2. Correlation between yield and nitrogen doses

Following the determinations made regarding the quality of the corn kernels, the protein content varied within quite large limits, from 8.7% in the variant in which the dose of fertilizer administered was $N_{120}P_{40}$, and the maximum (12.1% protein) was recorded in the $N_{120}P_{40}$ variant (Figure 3).
The highest protein content was obtained in the variant in which the dose of nitrogen administered was 120% active substance, on a phosphorus agrofond of 40 to 120%.

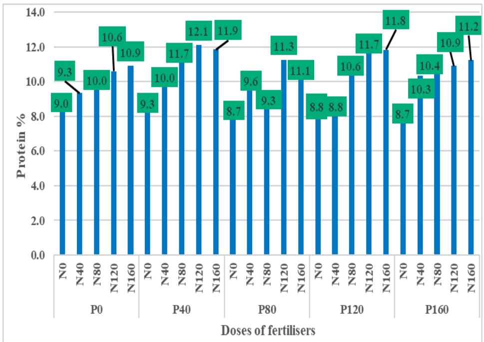

Figure 3. The protein content (%) of corn by applying fertilisers with nitrogen and phosphorus

The doses of fertilizers applied based on nitrogen and phosphorus had a very small influence on the content in the oil, thus the lowest value (3.5%) was obtained in the variant unfertilized (N_0P_0 - control) and the maximum of 4% oil content it was recorded in the variants for which the nitrogen dose was 40 respectively 80% on an 80% phosphorus agrofond (Figure 4).

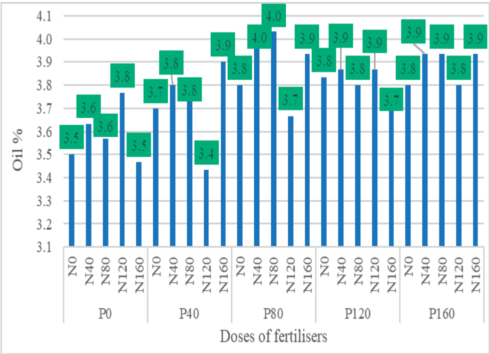

Figure 4. The oil content of corn by applying fertilisers with nitrogen and phosphorus

Regarding the starch content of corn, it varied from 70.1% in the variant in which the administered dose of fertilizer was $N_{120}P_{160}$ to 74.1% in the case of variant in which the administered dose was N_0P_{160} (Figure 5).

It can be concluded that nitrogen has an influence on the content in starch, because it decreases with the increase in the dose of fertilizer, both in the agrofond where no dose of phosphorus is applied, and in those where it was applied (Figure 5).

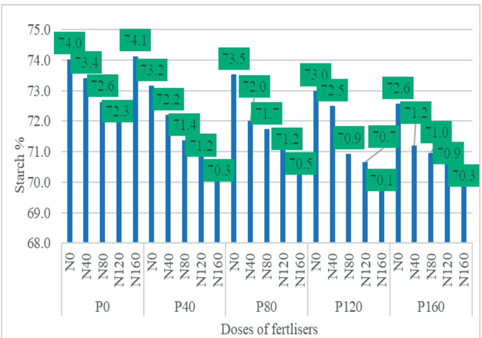

Figure 5. The starch content of corn by applying fertilisers with nitrogen and phosphorus

In average, in the ten years of testing, the values of the thousand kernel weight (TKW) at corn, varied within very wide limits from 271 grams at the variant with the dose of N_0P_{40} active substance, to 336.5 grams at the variant with $N_{160}P_{160}$ (Figure 6).

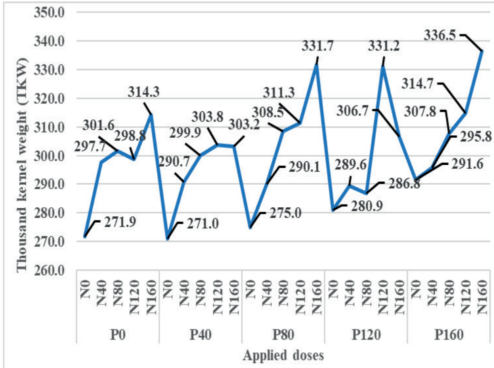

Figure 6. Influence of different doses of fertilisers on thousand kernel weight at corn (TKW)

CONCLUSIONS

In the ten years of research, the yields obtained in the corn crop recorded variations due to the combination of used fertilizers, the size of the dose administered but also the weather conditions, this explaining the very large differences in yield, from 1598 kg/ha ($N_{160}P_{80}$) up to 14065 kg/ha ($N_{160}P_{160}$).

On average, the yield obtained on the maize crop brought yield increases of 12 to 49% representing 731 to 3067 kg·ha^{-1}.

The interaction of phosphorus fertilizers on production brought increases of 4 to 12% (332-879 kg·ha^{-1}), and those with nitrogen increased by 12 to 29% (815-1979 kg·ha^{-1}).

Following the correlation between the obtained corn yields and the phosphorus and nitrogen doses applied, direct correlations were established between them, statistically ensured and interpreted as distinctly significant and very significant.

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THE RESEARCH METHODOLOGY USED REGARDING THE BEHAVIOUR OF SOME SORGHUM HYBRIDS IN TERMS OF PRODUCTIVITY AND ADAPTABILITY UNDER THE CONDITIONS AT BRAILA AGRICULTURAL RESEARCH AND DEVELOPMENT STATION

Florin MOCANU, Costică CIONTU

University of Agronomic Sciences and Veterinary Medicine of Bucharest,
Marasti Blvd 59, District 1, Bucharest, Romania

Corresponding author email: mocanuflorinn24@gmail.com

Abstract

The plant that was the object of the research was represented by the sorghum plant (Sorghum bicolor var. eusorghum) and the aim of the work was to be able to observe the behaviour of some hybrids in the pedo-climatic conditions of the Braila Agricultural Research and Development Station. It is known about grain sorghum that it presents some physiological characteristics that allow it to be cultivated in restrictive pedo-climatic conditions. These conditions refer to the presence of poor soils and areas where temperatures are higher than average and water supplies are poor. Under such conditions it is more difficult for the maize crop to provide economically profitable production. In this situation, growers can use sorghum as a safe option for obtaining profitable productions. In this paper it will be discussed the adaptability and productivity, two important elements for this crop.

Key words*: sorghum, productivity, adaptability.*

INTRODUCTION

The main representative of the *Sorgum* genus is *Sorghum bicolor*, which presents several varieties as well, the most well-known and used in culture being: *Sorghum bicolor* variety *eusorghum* (grain sorghum), *Sorghum bicolor* variety *technicum* (technical sorghum or broom sorghum), *Sorgum bicolor* variety *saccharatum* (sugar sorghum), and *Sorgum bicolor* variety *sudanese*, which is the forage sorghum (Roman Gh. V., 2011)

Sorghum is the cereal that occupies the fifth place in the world among the cultivated cereals, being surpassed only by wheat, rice, corn and barley, worldwide the area occupied by sorghum is 40 million ha., with the main cultivation areas on the African continent (Nigeria Sudan, Ethiopia), India but also important areas of the USA. From Europe, Russia, France, Spain and Italy stand out as countries with high areas cultivated with this plant. (https://www.sorghum-id.com/content/uploads/2022/02/2.bis-Arthur-Boy-Pierre Guillaumin.pdf)

What these areas have in common is the constant presence of high temperatures and low rainfall, giving sorghum plants an important place in the culture, being able to obtain very good yields where corn is limited by adverse pedological and atmospheric factors. This adaptability has given sorghum the nickname "The camel of crops". Also, through the research carried out in 2002 by Antohe , it was demonstrated that sorghum has a beneficial role in preventing land desertification.

Sorghum is a cereal with many uses, being successfully used in human food (African countries being an example in this sense) through the suitability of using flour in baking and pastry, the use of sweet syrup as a substitute for sugar and even obtaining drinks from sorghum grains. (Antohe, 2007; Bâlteanu, 2003; Pochișcanu et al., 2016; 2017). In the conducted researches, the role of sorghum in the medical field was highlighted due to the compounds the grains contain: ferulic acid, p-coumaric acid, p-hydroxybenzoic acid, and vanillic acid (Fatoki et al., 2023)

In animal nutrition, sorghum also has an important place considering that the use of sorghum grains mixed with corn grains, in a 50-50 percentage, has a special contribution, but also sorghum hay has a high value.

Correlating the fact that *Sorghum bicolor* presents good productions, varied uses in multiple branches of industry with the fact that areas in Romania, as well as worldwide, no longer benefit from the usual rainfall, sorghum can be a solution that can be counted on in the years that will follow.

MATERIALS AND METHODS

The research took place in the 2020-2021 and 2021-2022 agricultural years in Braila County on a chernozem soil, characteristic of the area moderately calcium-supplied in the upper part of the profile and strongly carbonated in the lower part (19.3%), with a medium humus content (2.4-3.1%) in the upper horizons and only 1.6 % in the transition horizon.

Total nitrogen content varies between 0.14-0.25% mobile phosphorus content 174-225 ppm and mobile potassium 24.0-26.0 mg/100 g soil in the arable layer and with a pH of 7.9-8.4. As physical and hydrophysical indices, the soil has an apparent density of 1.10-1.31 g/cm^3 with a field capacity of 22.9-25.2%, a wilting coefficient of 6.7-10.2%, a hygroscopicity coefficient of 3.7-6.4% and a minimum ceiling of active humidity of 13.8-17.4%.

The experiment was made by the method of subdivided plots and having 4 repetitions (in 2020-2021) and 3 repetitions for the 2021-2022 agricultural year.

In the experience, factor A was tested with 8 graduations a1 - Es Aize; a2 - Es Shamal; a3 - Es Arabesque; a4 - Es Foehn; a5 - Anggy; a6 - Ggustav; a7 - Belluga; a8 - Huggo.

All these hybrids are classified as hybrids with very low tannin content, less than 0.3%, starch content of 78% and protein content of 10-11%, being hybrids with high production capacity, especially in favorable conditions. (https://ragt-semences.fr/sites/default/files/public/medias/variety/pdfs/RGT%20ANGGY.pdf).

The factor B was represented by the sown density wich had 2 graduations: the density of 220.000 seeds/ha, and 250.000 seeds/ha with 70 cm between the rows for the firts density respectievly 50 cm between the rows for the second one.

The 2020-2021 agricultural year began with the preparation works of the agricultural land that had been cultivated with autumn wheat . These works carried out in the fall of 2020 were represented by harrowing.In the spring of 2021, starter fertilization was carried out with the DAP 18-46-0 complex fertilizer with an ammonia nitrogen content of (NH_4) - 18% and phosphorus pentaoxide (P_2O_5) - 46%. The dose applied was 200 kg per hectare of commercial product.

The next two works that were executed were the pre-emergent herbicide applying with the total herbcide Leo Green 360 with the active substance content of 360 g/l glyphosate in a dose of 4 l/ha and then the preparing of the germinal bed, corresponding to sowing.

The hybrids were sown on 26th of May 2021 in an experimental field that was made up of 8 variants (corresponding to each hybrid), with 4 repetitions each.

The plots consisted of six rows of sorghum 8 meters long, with a sowing distance of 70 cm between rows, at a density of 220.000 plants/ha and and the distance of 50 cm with the density of 250.000 plants/ha.

The harvest took place on 1st October 2021, being executed manually.

In the 2021-2022 agricultural year, the experiences with sorghum hybrids for grains were positioned on another land, an area that had corn as its predecessor plant.

It was harvested at the end of October 2021, after which the soil scarification work was carried out and then harrowing to break up the large soil formations.

In 2022, at the end of March, complex fertilizers 15-15-15 were administered to increase the content of N, P_2O_5 and K_2O.

Sowing was carried out on 8th of May 2022, being preceded by the preparation of the germinal bed.

After sowing, herbicide application was carried out with Barbarian Super and Dual Gold with the active substance glyphosate, respectively S-metolachlor to combat both monocotyledonous and dicotyledonous weeds.

A second fertilization work was carried out at the end of May, applying granular urea with a N content of 46%. In the case of treatments, Casper herbicide was also applied in June, with the active substances prosulfuron 5% and dicamba 50% to combat dicotyledonous weeds.

The harvesting took place on 15th of September 2021 when the sorghum hybrids reached the

optimal moisture of 15 %. The 2021-2022 agricultural year was characterized by the fact that precipitation was reduced compared to the previous year and compared to the multi-year average, so from the date of sowing to the date of harvest, the value of 125.5 mm/m² was recorded.
The collected data from the field were analysed using ANOVA function from Microsoft Word.

RESULTS AND DISCUSSIONS

In the therm of climatic conditions the data shown in the Table 1 highlighted the fact that the 2020-2021 agricultural year was characterized as a year with a high precipitation rate comapred to the multiannual. The temperature recorded was also increased than the multiannual.

Table 1. The climatic conditions registered in Braila Agricultural Research and Development Station in 2021

Month	2021 Temperature (°)	Multiannual average (°)	2021 Precipitations (mm)	Multiannual average (mm)	2021 Solar radiation	2021 Wind speed
January	2.2	-2.1	41.2	28	90.7	3.2
February	2.4	-0.2	7.4	27	90.5	2.8
March	4.7	4.7	31.4	26	150.9	2.8
April	9.4	11.2	53.3	35	170.0	2.4
May	16.7	16.7	75.8	48	213.6	2.2
June	20.2	20.9	173.8	62	181.1	3.1
July	24.8.	22.9	40.4	46	287.7	1.6
August	23.4	22.1	36.7	39	280.4	3.8
September	16.9	17.3	10.4	32	207.5	1.6
May-September	21.2	20.6	337.1	227	1170.3	2.4

The 2021 year was characterized as a year rich in precipitation, thus from January to September in five months the precipitation rate was higher than the multiannual for the same month. The total amount of precipitation for the May-September period , was also higher compared to the multi-year period corresponding to these months. During this period, the value of 337.1 mm was recorded, 110.1 mm more than the multi-annual May-September in this area of Braila and from the date of sowing to the date of harvest, the value of 304.4 mm/m² was registered. Sorghum, a plant with low demands in terms of water consumption, benefited from a sufficient amount, especially during the period of formation and filling of the grains leading to increased production elements. From the point of view of temperature, the year 2021 stood out for the fact that were registered higher values than the multi-annual average, thus, the average of the sorghum vegetation period recorded values higher than the multi-annual average by 0.6°C. Along with the climatic elements, the hybrid factor and the density factor also had a very important influence on the growth and development of sorghum plants. The morphological determinations studied were highlighted in Table 2. Those were the plant height, panicle length and the number of leaves. The results show that both the density of the plants per hectare and the hybrid that has been used influenced these parameters. Depending on the density, the values of the plant height were in the range 117.0 cm and 120.0 cm, the panicle length were situated in 23.1 cm to 24.9 cm and the leaf number was situated between 7.8 and 8.3 leaves per plant. The lower values are correspondent for the higher plant density. From the point of view of the hybrid that has been used the lowest plant height is present at the Belugga hybrid. It registered 95.1 cm for the 220.000 seeds/ha density and 90.6 cm for the higher plant density. The biggest height recorded was observed at the Es Foehn hybrid both at the density of 220.000 and 250.000 seeds/ha. In the term of panicle length the biggest value was measured at the Es Arabesk variety and that was 26.6 cm, being present at the 220.000 density. At the 250.000 seed density the length of 25.3 cm stood out as the highest panicle length. The biggest leaf number was observed on the Es Foehn variety at the lower density.

Table 2. The influence of density and hybrid on the sorghum development in 2021

Sowing density (Seeds/ha)	Hybrid	Plant Height (cm)	Panicle lenght (cm)	Leaf number/plant
Density 1-220.000	Es Alize	117.5	22.7	8.4
	Es Shamal	122.7	24.7	8.5
	Es Arabesk	124.3	26.6	8.5
	Es Foehn	130.1	26.2	9.2
	Anggy	128.1	25.2	8.3
	Ggustav	119.7	26.4	7.9
	Belugga	95.1	22.5	7.3
	Huggo	124.9	25.0	8.4
Average		**120.3**	**24.9**	**8.3**
Density 2-250.000	Es Alize	115.1	20.0	7.3
	Es Shamal	119.3	22.9	8.0
	Es Arabesk	122.1	24.4	8.1
	Es Foehn	126.3	25.3	8.4
	Anggy	125.6	23.5	8.3
	Ggustav	116.8	24.0	7.5
	Belugga	90.6	21.5	7.1
	Huggo	120.0	23.4	7.8
Average		**117.0**	**23.1**	**7.8**

The experience sustained in 2021 confirmed the adaptability and productivity of grain sorghum. In the location of the experiment, the Brailei Plain sorghum for grains obtained important yield, without requiring an additional amount of water from irrigation, the pluviometric input ensuring the crop's needs however the fertilization regime used consisted in 35 kg N/ha and 90 kg P/ha. In the Table 3 and Figure 1 are registered the productions obtained by the eight hybrids at the two densities.

Table 3. The influence of density and hybrid on the sorghum yield in 2021

Sowing density (Seeds/ha)	Hybrid	Grain yield			Signification
		kg/ha	%	Differences (kg/ha)	
Density 1-220.000	Es Alize	6654	93.2	-485	-
	Es Shamal	7880	110.4	741	-
	Es Arabesk	6047	84.7	-1092	o
	Es Foehn	7229	101.3	90	-
	Anggy	8212	115.0	1073	*
	Ggustav	8628	120.9	1489	**
	Belugga	7378	103.3	239	-
	Huggo	6441	90.2	-698	-
Density 2-250.000	Es Alize	6318	88.5	-821	-
	Es Shamal	6640	93.0	-499	-
	Es Arabesk	5945	83.3	-1194	o
	Es Foehn	6551	91.8	-588	-
	Anggy	7892	110.5	753	-
	Ggustav	8595	120.4	1456	**
	Belugga	7242	101.4	103	-
	Huggo	6569	92.0	-570	-
Experience average-Control		7139	100	Control	Control

LSD 5% - 890.5 (kg/ha), LSD 1% - 1315.1 (kg/ha), LSD 0.1% - 2032.7 (kg/ha)

From the point of view of the hybrid factor, the average production obtained recorded the value of 7139 kg/ha, the highest being recorded by the hybrid Ggustav, namely 8628 kg/ha under the conditions of the density of 220.000 germinating seeds/ha. From the point of view of the density factor, at Density 1 the average production of hybrids was 7309 kg/ha and for Density 2 it was 6969 kg/ha.The Arabesk hybrid recorded a value of 6047 kg/ha at the lower density and 5945 kg/ha at the higher density. These two values show negative statistical significance. The hybrid Anggy with the production of 8212 kg/ha at Density 1 is significantly positive and the hybrid Ggustav with productions of 8628 kg/ha at the density of 220.000 plants per hectare and 8595 kg/ha at 250.000 plants per hectare is classified as distinctly significant positive.

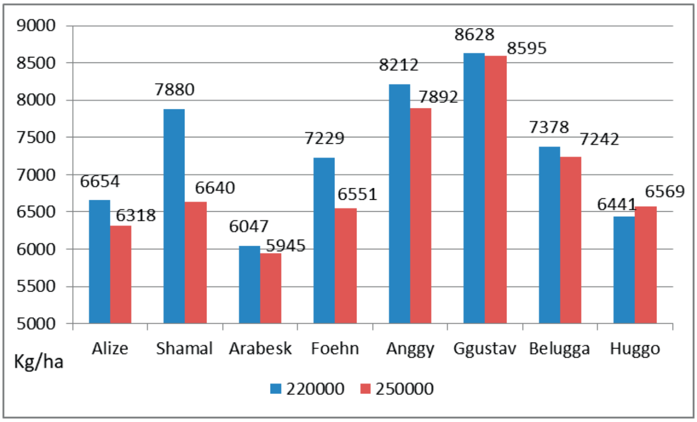

Figure 1. Influence of the density and hybrid on sorghum yield in 2021

The year 2022 stood out from the point of view of the pronounced atmospheric drought, so in Table 4 it can be seen that in each month of the year, the recorded precipitation is lower compared to the multi-annual specific for the same month. For the period May-September, the vegetation period of sorghum, it can be observed that the precipitation totals 125.5 mm, being 101.5 mm less than the multi-annual specific for this period. From the point of view of the recorded temperature, for each month of 2022 the value compared to the multi-annual is higher, with the exception of March. For the May-September period, a higher value was recorded by 1.0°C higher than the multi-annual average of the same period.

Table 4. The climatic conditions registered in Braila Agricultural Research and Development Station in 2022

Month	2022 Temperature (°)	Multiannual average (°)	2022 Precipitations (mm)	Multiannual average (mm)	2022 Solar radiation	2022 Wind speed
January	1.3	-2.1	6.5	28	114.3	2.5
February	4.1	-0.2	11.1	27	126.5	3.0
March	3.8	4.7	13.8	26	171.8	3.0
April	11.9	11.2	25.1	35	193.0	3.0
May	18.0	16.7	24.3	48	269.0	2.1
June	22.7	20.9	33.3	62	229.7	2.0
July	24.8	22.9	8.9	46	281.9	2.1
August	24.9	22.1	26.9	39	246.1	2.1
September	17.9	17.3	32.1	32	215.2	2.4
May-September	21.6	20.6	125.5	227	1241.9	2.1

It can be observed in Table 5 the morphological determinations made during the experience with sorghum in the year 2022. For the density factor, the lower values are recorded for Density 1, thus the height of the plant is 106.8 cm, higher than the 105.2 cm specific to Density 2. The height of the panicle records higher values in the case of the density of 220,000 seeds/ha. Also the number of leaves is slightly higher in the case of Density 1, namely 7.6, compared to 7.5 for the higher density. From the point of view of the hybrid factor, Ggustav has the highest height: 114.6 and the longest panicle: 25.9 cm. The Foehn hybrid has a superior value for the number of leaves: 8.1.

Table 5. The influence of density and hybrid on the sorghum development in 2022

Sowing density (Seeds/ha)	Hybrid	Plant Height (cm)	Panicle lenght (cm)	Leaf number/plant
Density 1-220.000	Es Alize	101.3	22.3	7.5
	Es Shamal	108.4	22.5	7.6
	Es Arabesk	105.5	24.7	7.5
	Es Foehn	111.8	24.4	8.1
	Anggy	115.1	24.0	7.9
	Ggustav	115.5	26.7	7.2
	Belugga	87.0	21.8	7.0
	Huggo	109.8	22.4	7.8
Average		**106.8**	**23.6**	**7.6**
Density 2-250.000	Es Alize	99.6	21.1	7.3
	Es Shamal	106.5	21.9	7.4
	Es Arabesk	104.1	23.9	7.4
	Es Foehn	109.9	23.6	8.2
	Anggy	114.0	23.8	7.7
	Ggustav	113.7	25.2	7.0
	Belugga	85.8	21.6	6.9
	Huggo	108.1	22.7	7.7
Average		**105.2**	**23.0**	**7.5**

Table 6. The influence of density and hybrid on the sorghum yield in 2022

Sowing density (Seeds/ha)	Hybrid	Grain yield			Signification
		kg/ha	%	Differences (kg/ha)	
Density 1-220.000	Alize	5059	89.8	-573	o
	Shamal	5624	99.9	-8	-
	Arabesk	4977	88.4	-655	oo
	Foehn	5807	103.1	175	-
	Anggy	6088	108.1	456	*
	Ggustav	6544	116.2	912	**
	Belugga	5717	101.5	85	-
	Huggo	5804	103.1	172	-
Density 2-250.000	Alize	4630	82.2	-1002	ooo
	Shamal	5109	90.7	-523	o
	Arabesk	4988	88.6	-644	oo
	Foehn	6017	106.9	385	-
	Anggy	5955	105.8	323	-
	Ggustav	6251	111.0	619	*
	Belugga	5720	101.6	88	-
	Huggo	5817	103.3	185	-
Experience average-Control		5631	100	Control	Control

LSD 5% - 423.9 (kg/ha), LSD 1% - 626.1 (kg/ha), LSD 0.1% - 967.6 (kg/ha)

In 2022, a year in which there was no favorable rainfall regime, the sorghum hybrids obtained an average production of 5649 kg/ha. The productions obtained fell within the limits of

4630 kg/ha and 6544 kg/ha as can be seen in Table 6 and in Figure 2. Depending on the hybrid factor the highest value was obtained by the Ggustav hydride, which obtained a average of 6533 kg/ha. Taking into account the density factor, it is deduced that Density 1 brings a higher production compared to Density 2, thus at 220,000 seeds/ha an average production of 5703 kg/ha is obtained, and for 250,000 seeds/ha the average production is 5595 kg/ha. From a statistical point of view, it can be observed the Ggustav hybrid, which at Density 1 obtains a distinctly significant positive production and the Es Alize hybrid, at a density of 250,000 seeds/ha obtains a very significant negative value. Most of the variants are statistically insignificant.

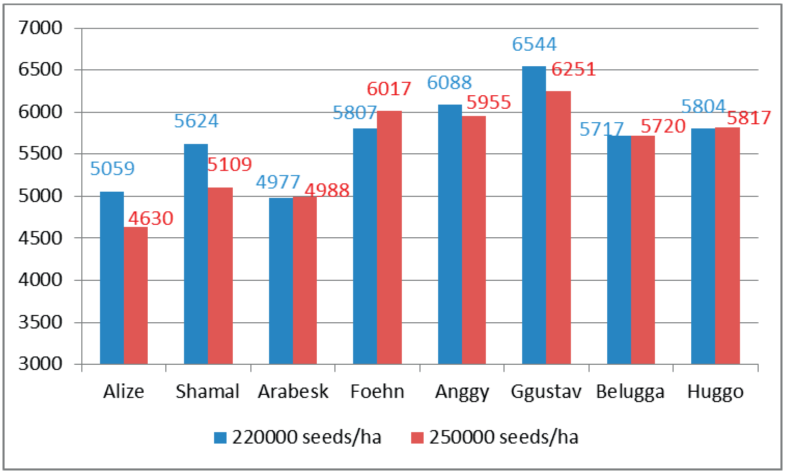

Figure 2. Influence of the density and hybrid on sorghum yield in 2022

CONCLUSIONS

Taking into account what is presented in this paper, the following aspects can be highlighted: The experience with sorghum hybrids took place over the period of two years, namely 2021 and 2022. Among them, the year 2021 was a year that ensured a significant rainfall regime, thus during the sorghum vegetation period, namely May-September, a amount of 337 mm precipitation. In these atmospheric conditions the average production of the experiment was 7139 kg/ha. The Ggustav hybrid was the one that ensured the maximum value of 8628 kg/ha at the density of 220.000 seeds/ha. The fertilization regime that was used was represented by the application of 35 kg N/ha and 90 kg P/ha. The year 2022 was dry and warmer than the multi-year average. This year the production average was 5631 kg/ha, with the maximum production obtained of 6544 kg/ha, of the same Ggustav hydrid, also at the density of 220.000 seeds/ha. For the year 2022, 130 kg N/ha and 40 kg P/ha were applied for fertilization. The climatic differences between the two years had an important impact, thus the productions recorded in 2022 were much lower compared to the previous year. From the productions obtained, the density factor had an important role, thus the lower density ensured higher values. All eight commercial hybrids showed superior yields.

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OPTIMAL DOSES AND CONCENTRATIONS OF MUTAGENS FOR WINTER WHEAT BREEDING PURPOSES. GRAIN QUALITY

Mykola NAZARENKO, Oiexander IZHBOLDIN, Inna LIADSKA, Nataliia PASHCHENKO

Dnipro State Agrarian and Economic University, 25 S. Efremova, Dnipro, Ukraine

Corresponding author email: nik_nazarenko@ukr.net

Abstract

In addition to increasing grain yield, a promising direction in the use of mutagens is to obtain complex changes that increase the content and quality of grain protein. The main purpose of investigation is to develop possibilities of difference mutagen agents in induction mutants by protein content and quality, bread-making technological qualities and pathways for obtaining forms with combination of good grain production and qualities parameters. From 13 perspective high grain productive mutant lines 3 were separated out by combination of good grain quality and grain productivity on the level no less than national standard. Medium dose of gamma-rays (100 Gy) and concentrations of nitrosomethilureas (0.0125%) are effectively for mutation breeding on grain productivity and quality in complex. It is planned to conduct research for the optimal doses and concentrations of mutagens to obtain forms which are tolerant to abiotic stresses.

***Key words**: grain quality, mutagenesis, mutation breeding, protein content, winter wheat.*

INTRODUCTION

Induced mutagenesis is well recognized as one of the key technology for the development of new varieties by all agronomic-value traits. Induced mutations have been applied to produce mutant varieties by changing the plant characteristic for a significant increase in production and improve quality (Shu et al., 2013).

Much excitement was generated as novel mutants overcame major obstacles in crop improvement and/or produced new and valuable variants. New forms such as semi-dwarfism, early maturity, disease resistance, etc. met immediate market demands and were often released directly as commercial varieties without recourse to refinement through cross breeding (Per et al., 2016). The development of direct mutants into commercial varieties is still a common practice in seed propagated crops. Mutation breeding gave an initial material for green revolution (Ahloowalia et al., 2004).

The most part of mutant varieties have been obtained by using gamma-irradiation (about 85 percent from general number) (Ahloowalia et al., 2004). Mutants generated through induced mutagenesis have been also used in genetics studies (van Harten, 1998; Waugh et al., 2006). Plant induced mutagenesis has been used widely as a tool in basic studies and practical breeding programs, it is seldom considered to be an independent subject by plant scientists or plant breeders (Jankowicz-Cieslak et al., 2017). Improvements in plant breeding can only be made when sufficient variation for a given trait is available to the breeder (Cheng et al., 2015).

Wheat is the top food crop in Ukraine. The total area for winter wheat cultivation in Ukraine covers 6.8 million hectares with actual total productivity of 24 million tons and average yield of 3.8 t/ha (Nazarenko, 2016a). Most modern wheat mutant varieties are not direct mutants, but a product of additional breeding (Chazav et al., 2010). One of the controversial questions of wheat mutation breeding is the problem of mutagens doses and/or concentrations suitable for creation new value genotypes (contradictory between high (Mangi et al., 2016) and medium dose usage (Shu et al., 2013; Nazarenko, 2016b).

The increase of overall collection of grain is, obviously, one of the priorities, but the grain must also be of quality. Wheat grain is still the main source of protein and minerals; it has an optimal balance of protein and starch. That is, the grain quality combined with other properties of economic value is the required characteristic (Kenzhebayeva et al., 2018).

Grain quality character polygenic nature and high modification variability typical of it provide additional difficulties at selection by

quality of created mutant lines (Taulemesse et al., 2016).
The selection by grain quality characteristic is also complicated by the great dependency of functional properties of grains on the conditions of their handling, which becomes a significant problem when creating a genotype that combines high quality and high-yielding ability (Albokari, 2014; Aamir et al., 2016; Jaradat, 2018).
Mainly, the proteins of protein fractions, which are difficult to dilute (globulins, prolamins), are involved in the process of creating a high-quality grain. Such fractions of proteins are synthesised by membrane-bound ribosomes. The hydrophobic (membrane) environment promotes the synthesis of protein within scaled-up hydrophobic properties, namely the high quality proteins. A temperature drop and treatment with sodium chloride result in the destruction of hydrophobic bonds and synthesis of highly soluble proteins of lower quality. The varieties, which are characterised by a steady state of the protein synthesis systems under all conditions, synthesise mainly the protein fractions that are difficult to dilute (Kang and Banga, 2013).
The aim of the investigation is to determine optimal dose and concentrations of mutagens for mutation breeding practice and obtain new mutants with combinations of high-grain yield and quality, methods of identification of new mutants among the mutant population.

MATERIALS AND METHODS

Dried wheat grains (approx. 14% moisture content, in brackets method of obtaining varieties or used mutagens) of 'Favoritka', 'Lasunya', 'Hurtovina' (irradiation of initial material by gamma rays), line 418, 'Kolos Mironovschiny' (field hybridization), 'Sonechko' (chemical mutagenesis, nitrosodimethilurea (NDMU) 0.005%) and 'Kalinova' (chemical mutagenesis, 1,4-bisdiazoatsetilbutan DAB 0.1%), 'Voloshkova' (termomutagenesis - low plus temperature at plant development stage of vernalizaion has been used as mutagen factor) of winter wheat (*Triticum aestivum* L.) were subjected to 100, 150, 200, 250 Gy gamma irradiation (acid dose, Co60, 0.048 Gy/s) and treated with solutions of chemical mutagens - nitrosomethilurea (NMU) 0.0125 and 0.025%, nitrosoethilurea (NEU) - 0.01 and 0.025%, dimethylsulfate (DMS) - 0.0125, 0.025 and 0.05%, 1,4-bisdiazoatsetilbutan (DAB) - 0.1 and 0.2% (grains were soaked in mutagen solution). Each treatment was comprised of 1,000 wheat seeds. Exposition of chemicals mutagens was 18 hours (Nazarenko, 2017). These concentrations and exposure are trivial for the breeding process that has been repeatedly established earlier. Non-treated varieties were used as a control for mutation identified purpose.
In M2 - M3 generations agronomic-value families (by protein content) have been selected. Estimation of protein and its components was conducted from 2014 to 2018 years (M4 - M8 generations). The controls were national standard 'Podolyanka' and initial varieties. The trial was set up as a randomized block design method with three replications and with a plot size of from 5 to 20 m^2 in 2-3 replications. Total size of field trial at M2 - M3 generations was 53,450 families (include controls).
The mutant lines were processed during the period of grain filling (X-XI stages of organogenesis) with the substances that change the state of protein synthesizing systems as a method for protein quality identification (sodium chloride in concentration of 5 g/l). The bread-making characteristics were identified for lines too (2016-2017 fields seasons). Total size of field trial was 148 lines.
Wheat samples were held at room condition at 18 - 20 for several days before grinding. Each sample of 30 g weigh was separately ground on a laboratory cyclone grinder (LMT-1, PLAUN LLC, Russia). Protein, gluten and water content of the samples were measured by Near-infrared Reflectance Spectroscopy (Spectran-IT, CJSC, Russia). Triplicate data of each sample were averaged. Contents of gliadin and glutenin were identified on CNS Model Flash EA 1112 (for protein content) and RP-HPLS (for gliadins and glutenins).
Experiments were carried out on the experimental fields of Dnipro State Agrarian and Economic University. The field`s geographic coordinates are: 48°30'N lat. and 35°15' E long. The experimental field is lied on 245 meters above the sea level. The air temperature during winter wheat growing

season (September - July) is 8-11°C, the average rainfall is about 350-550 mm in similar vegetation season.
Mathematical processing of the results was performed by the method of analysis of variance, the variability of the mean difference was evaluated by Student's t-test, cluster and correlation analyses was conducted by module ANOVA. In all cases standard tools of the program Statistica 8.0 were used.

RESULTS AND DISCUSSIONS

Table 1 summarises the data of investigations of the second- and third-generation mutant families on high protein content mutations rate. It can be seen from the table that the rate of this type of mutation was not as high as for changes in grain yield. For most of the variants, this mutation was rare (not more than 0.2-0.4%). For only two variants it was 1.0 % and average by frequency of occurrence. According to the previous investigations (Singh and Balyan, 2009), the rates of this type of mutants did not exceed 0.4-0.6% on average and reached 1.2- 1.4% for some variants with optimal genotype and mutagen factor com-bination. The rate generally depended on the nature of mutagen, the dose or concentration of mutagen and genotype.
Regarding ANOVA analysis, the level of factors corresponded to the nature of mutagen (F = 10.80; Fcritical = 3.22: p-level 0.01), for genotype (F = 4.34; Fcritical = 2.96: p-level 0.01), for dose or con-centration (F = 3.18; Fcritical = 3.11: p-level 0.01).

Table 1. Rates of mutations by grain quality after mutagen action

Trial	Kolos Mironivschini	Kalinova	Voloshkova	Sonechko	Favoritka	Hurtovina	Lasunya	Line 418
Control	0	0	0	0	0	0	0	0
Gamma-rays, 100 Gy	0.4	0.4	0.8*	0.4	0.6*	0.8*	0.2	0.2
Gamma-rays, 150 Gy	0.2	0.2	0.4	0.4	0.6*	0.6*	0.2	0.2
Gamma-rays, 200 Gy	0.2	0	0.2	0	0	0.2	0	0
Gamma-rays, 250 Gy	0.2	0.2	0.4	0	0.2	0.2	0	0
NMU 0.0125%	0.6	0.6*	0.6*	0.4	0.4	0.6*	0.2	0.6*
NMU 0.025%	0.4*	0.6*	0.4	0.2	0.6*	0.4	0.2	0.6*
NEU 0.01%	0.8	0.2	0.8*	0.2	0.6*	1.0*	0.4	1.0*
NEU 0.025%	0.6	0.2	0.8*	0.2	0..8*	0.6*	0.4	1.0*
DAB 0.1%	0	0.2	0	0.2	0	0	0	0.2
DAB 0.2%	0.2	0	0.2	0.2	0.2	0.2	0	0.2
DMS 0.0125%	0.4	0.4	0.2	0.4	0.4	0.2	0.2	0.4
DMS 0.025%	0.2	0	0	0.2	0	0.2	0	0.2
DMS 0.05%	0	0.2	0	0.2	0	0	0.2	0

*statistically significant differences from standard at $P_{0.95}$

As for mutagen nature, more successful for this type of mutations was the gamma-radiation of 100 Gy (less, but also suitable, 150 Gy) - 0.4-0.8%, nitrosoalkylureas (NEU more successful than NMU) induced mutations up to 1.0%, without statistically reliable difference between the concentrations for both muta-gens.
Gamma-rays irradiation was more suitable for the varieties of Voloshkova, Favoritka, Hurtovina only (0.6-0.8%), and chemical exposure was more effective for the genotypes of Kolos Mironivschini, Voloshkova, Favoritka, Hurtovina, line 418. DMS and DAB were not effective for this type of mutation induction for any concentrations and any genotypes.
Two lawlike regularities can be observed. Mutagens were more effective in induced mutation by protein content, primarily for varieties with low protein content than for high-protein varieties (e.g. Sonechko and Lasunya). Mutagens have proved more effective in genetic alterations for varieties with non-mutational origins (line 418).
It has been observed that most high-protein mutations were associated with changes in spike morphology (Nazarenko, 2016) and in grains shape, which is consistent with the previous investigations into mutation breed for crop improvement, but for this type of complex mutations, the level of grain production was

unsatisfactory and these forms were useless as a future commercial variety.

The objective of the second stage of the research was to assess the quality of grain with multiple parameters of a well-known variety (as the standard) (Kang and Banga, 2013), and then new mutant lines using the substances that destroy hydrophobic bonds. The analysis of protein-synthesising systems of 14 lines and the standard variety "Podolianka" was carried out. The content of wet gluten was determined by Kjeldahl method using Sereniev's devices.

The lines were processed during the grain-filling stage with the substances that alter protein-synthesising systems (sodium chloride in a concentration of 5 g/l).

The previous research has shown a decrease in total protein content for high-quality varieties after spike treatment during the grain-filling stage with the substances reducing the amounts of membrane-bound ribosomes.

After the treatment of winter wheat at the X-XI stages of organogenesis with the substances that break the hydrophobic bonds, the Podolyanka variety in lines 157 and 213 showed an increase in protein content (Table 2). This indicates poor grain quality. In other lines, there was no response to the exposure. For these lines, the protein processing is more related to free ribosomes, and they have not reacted to the influence of the agent breaking ionic bonds, indicating their stability in terms of grain quality. Of particular note are lines 133, 156, 174, which have excellent technological characteristics of flour.

Table 2. Influence of substances, which destroyed hydrophobic relations on protein content and other technological parameters of winter wheat mutation lines grain

Line	Protein content, %		Technological parameters of quality		
	Control	Treatment	Sedimentation, ml	Gluten, %	Volume of bread for 100 g floura
Podolyanka	13.3±0.04	14.0±0.06	71	30.3	620
130	14.1±0.08*	12.6±0.08	49	23.3	660
133	13.5±0.03	13.2±0.05	54	25.1	590
142-1	13.2±0.09	13.6±0.1	36	22.2	470
156	14.3±0.04*	12.8±0.09	49	30.2	560
157	13.5±0.03	13.9±0.08	59	30.5	520
157-1	13.5±0.08	13.4±0.04	55	29	620
172	13.7±0.07	13.9 ±0.03	61	30.4	560
174	14.2±0.05*	11.3±0.05	51	27	620
179	13.0±0.06	13.2±0.07	53	26.8	640
185	13.2±0.02	13.4±0.03	76	27	610
186	14.2±0.07*	13.8±0.04	68,5	31	630
211	13.9±0.01*	13.5±0.5	64	24.5	570
213	13.9±0.10*	14.5±0.01	60	27.1	630

*statistically significant differences from standard at $P_{0.95}$

The analysis of correlations showed that the content of gluten is largely related to the flour sedimentation (0.51) and the percentage of protein in grains, obtained by plotting with the substances destroying hydrophobic bonds (0.57), and the volume of bread correlates with a total appraised value to the total score of 0.7.

The two-year analysis of the grain quality has confirmed the available data. The grain yielding capacity is backward dependent on the flour sedimentation -0.71, as well as on the increase of protein content -0.73.

The increase in protein content, with regard to the reference data, also has a positive correlation with grain swelling in water for 24 hours (0.77), and a negative correlation with yield of -0.67.

Table 3 summarises the dates by grain productivity (Nazarenko et al., 2018) and quality. Within the framework of this breeding program, 3 lines combining high yields and sufficient grain quality have been obtained. One of the lines, 156, has excellent baking qualities as does the complex. As for mutagens for two mutant lines, NMU 0.0125% has been used as a mutagen factor, for one 100 Gy. NEU, which was effective on a high level for induction of high-protein mutations in general, does not give a final result in new perspective lines obtained. Likewise for NMU, this case for this mutagen according to previous investigations. According to previous investigations, gamma-rays have to be used at low doses.

Table 3. Origin and describe of main morphological traits of winter wheat mutant lines

Line	Initial variety	Mutagen	Protein	Gliadin	Glutenin
Podolyanka (standard)	--	--	13.3	0.024	0.61
130	Kalinova	100 Gy	14.1*	0.031*	0.72*
156	Kolos Mironovschiny	NMU 0.0125%	14.3*	0.033*	0.74*
211	Kolos Mironovschiny	NMU 0.0125%	13.9*	0.024	0.71*

*statistically significant differences from standard at $P_{0.95}$

All 3 lines have a higher content of gliadins and glutenins than the standard Podolyanka and, the high content of these ingredients is thought to be indicative of the potentially high quality of grain and can be used as a complex parameter for breeding material evaluation.

CONCLUSIONS

Thus, gamma rays and nitrosolalburea can be used as mutants to induce mutation in grain quality. In all cases, DMS and DAB as mutagens are useless for these purposes. Mutants with high protein content were rare and often associated with lower grain yields due to changes in spike structure. For the gamma-rays, the more effective doses were in the interval of 100-150 Gy, while the prevalent ones were 100 Gy.

In spite of grain productivity mutations in previous studies, the frequency of high-quality grain mutations depends on the interaction between the genotype and the nature of the mutagen. The action of the corresponding mutagenic factor (gamma-rays, NMU) entirely determined the number of mutations by grain quality.

Our research has shown that the protein content in potentially high-quality lines decreases after the spike treatment while the grain is filled with the proposed solution, which lowers the number of membrane-bound ribosomes. The status of the protein-synthesising system of 13 mutant lines has been described. The dependence of hydrophobic bonds activity and protein quality allowed us to identify 3 high-quality mutant lines (133, 156, 174). In addition, 3 lines have been determined by a complex of high grain yield and good baking quality (mutant agents 100 Gy and NMU 0.0125%).

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ASSESSMENT OF LEAF RUST (*P. recondita* f. sp. *secalis*) ATTACK IN MARGINAL AREAS FROM SOUTHERN ROMANIA

Mirela PARASCHIVU[1], Otilia COTUNA[2, 3], Veronica SĂRĂȚEANU[2], Gheorghe MATEI[1], Reta DRĂGHICI[4], Alina Marinela PRIOTEASA[1]

[1]University of Craiova, Faculty of Agronomy, 19 Libertatii Street, Craiova, Romania
[2]University of Life Sciences "King Mihai I" from Timisoara, 119 Calea Aradului Street, Timisoara, Romania
[3]Station of Research and Development for Agriculture Lovrin, 200 Principala Street, Lovrin, Romania
[4]Research and Development Station for Plant Culture on Sands, Dabuleni, 217 Petre Banta Street, Dolj County, Romania

Corresponding author email: veronica.sarateanu@gmail.com

Abstract

Worldwide abiotic stress factors such as excessive temperature, precipitation, drought, salinity, soil pH, greenhouse gases, ultraviolet (UVB) radiation, and air pollution pose a persistent threat to both diseases and plants affecting host-pathogen relationship depending on geographical and temporal distribution of inoculum amount and cultivars susceptibility. Leaf rust of rye, which is caused by Puccinia recondita f. sp. *secalis (Roberge ex. Desmaz) has become one of the most important limiting factors for rye production in Central and Eastern Europe. During 2020-2021 growing season, a plant-pathogen interaction profile was observed on four rye genotypes in a randomized complete block design with three replications in dry area from Research and Development Station for Plant Culture on Sands Dăbuleni in south of Romania. Adult plant partial resistance was assessed through host response and epidemiological parameters as final rust severity (FRS), area under the disease progress curve (AUDPC), relative area under the disease progress curve (rAUDPC), coefficient of infection (CI) and infection rate (IR). The response of rye genotypes to leaf rust included different variation in resistance reaction ranging from moderately resistant to very susceptible. A negative and highly significant correlation of AUDPC with grain yield (r = -0.9222***) was found during 2020-2021 cropping season.*

Key words: *leaf rust, adult plant partial resistance, Puccinia recondita* f. sp. *secalis, epidemiological parameters.*

INTRODUCTION

Climate change is affecting many aspects of our world, including agriculture and horticulture (Howden et al., 2007; Cichi et al., 2008; IPCC, 2013, Velea et al., 2021; Bonciu, 2022a; Răduţoiu and Stan, 2022).
Cereals are one of the most important crops worldwide and they are vital for human consumption and animal feed (Neupane et al., 2022).
To feed the estimated 9.8 billion people on the planet by 2050, the supply of cereal food must rise by 70-100% and 2-3% (annually) (Godfray et al., 2010; Hawkesford et al., 2013; Ray et al., 2013; Tripathi et al., 2016). According to previous studies, over the next 30 years, the earth's average surface temperature will rise at a rate of about 0.2 degrees Celsius every decade (Solomon, 2007; Bernstein et al., 2008). There are several possible strategies including breeding, technical progress and improving fertilizer and pesticides efficiency to increase crops production (Bălașu et al., 2015a; Ză lă et al., 2023; Sălceanu et al., 2022). The conservation of genetic resources in agriculture and food security is a long-term challenge that transcends the borders of national interests (Paraschivu et al., 2017; Cichi and Cichi, 2019; Bonciu, 2020; De Souza and Bonciu, 2022a; De Souza and Bonciu, 2022b). Maintaining access to safe, disease- and pest-free and affordable agricultural products and raw materials and ensuring sustainable agricultural production are challenges that must be faced in the context of increasing demand for agricultural products (Bonciu et al., 2021; Bonciu, 2022b).
However, despite many changes in agricultural systems as technical progress, income growth, genetically progress, improved cropping

technologies, globalization on food production, machinery revolution, climate change is having a significant impact on the growth and health of cereal crops and the pathogens that affect these crops are also being affected (Partal et al., 2013; Partal et al., 2014; Cristea et al.,2015a; Cristea et al., 2015b; Paraschivu et al., 2015; Cotuna et al., 2018; Partal and Paraschivu, 2020; Păunescu et al., 2021; Paraschivu et al., 2022; Păunescu et al., 2022).

One of the most significant impacts of climate change on cereals is the increase in temperature (Chakraborty and Pangga, 2004; Chakraborty, S. & Newton, 2011; Chakraborty, 2013). Higher temperatures can lead to heat stress in plants, which can result in reduced yields and increased susceptibility to diseases (Coakley et al., 1999; Paraschivu et al., 2019a; Paraschivu et al., 2019b). For example, a 1°C increase in temperature during wheat cultivation could result in a 3-10% decrease in crop yields (Yao et al., 2012). This is because pathogens, such as fungi and bacteria, thrive in warm and moist conditions, and can infect plants more easily when they are stressed.

Another impact of climate change on cereals is the change in precipitation patterns. Changes in rainfall and humidity levels can lead to changes in the distribution and severity of plant diseases. For example, increased rainfall can create conditions that are conducive to fungal diseases, such as Fusarium head blight, which affects wheat and barley crops (Cotuna et al., 2013; Paraschivu et al., 2014; Cotuna et al., 2022b). This disease can lead to reduced yields and poor-quality grain, which can have a significant impact on farmers and the food supply. Also, climate change together with human-induced changes is expected to cause the spread of pathogens, pests and invasive species in areas where they have not been relevant before, bringing new challenges for crop management and breeding in order to face yield losses and avoid alteration of natural landscape vegetation (EEA Report, 2017; Răduţoiu D., 2020; Răduțoiu & Băloniu, 2021; Răduţoiu, 2022; Răduţoiu & Ștefănescu, 2022). Thus, some pathogens tend to become more aggressive even in cropping systems based on crops diversification by minor cereals.

Puccinia recondita f. sp. *secalis* (Prs) is a fungal pathogen that causes leaf rust in barley and wheat, but little is known about its host range and pathogenicity on rye.

Rye (*Secale cereale*) is a minor cereal, closely related to barley and wheat, used for human consumption as rye bread and alcoholic beverages, such as beer, whiskey and vodka and as feed for livestock. Currently rye crop contributes to crop species diversity in temperate regions of Central and Eastern Europe, especially in marginal environments where soil and climate are unfavourable for wheat production.

One of the most important diseases of rye in Central and Eastern Europe is Brown rust (BR), known also as Leaf rust (LR), caused by the obligate biotrophic basidiomycete *P. recondita* f. sp. *secalis* (Prs) (Roberge ex. Desmaz) (Roux et al., 2007; Roux and Wehling, 2010; Meidaner et al., 2012). Yield losses can be up to 40% in natural conditions, but they can be as high as 80% in case of early infection (Solodukhina, 2002; Wehling et al., 2003). This mainly happens to the pathogen`s ability to multiply rapidly, as well as to its air borne dispersal mechanism from one field to another (Brown and Hovmøller, 2002).

Developing resistant varieties of rye is an effective way to manage leaf rust. This approach is environmentally friendly and can be a cost-effective way to manage the disease (Singh et al., 2005). However, cereal rusts exhibit considerable capacity for generating, recombining and selecting for resistance under the impact of climate variability and they can adapt to new environment. Therefore, screening rye cultivars for adult plant resistance or using the marker-assisted selection in rye breeding program is of great importance to find new resistance genes associated with leaf rust resistance.

The present paper emphasises the results of the assessment of four rye genotypes, with different origins, screened for adult plant partial resistance to *P. recondita* f. sp. *secalis* in natural infections in the sandy soils southern Oltenia, Romania.

MATERIALS AND METHODS

With no molecular markers or differential sets available for the identification of races in *Puccinia recondita* f. sp. *secalis* (Prs), during

2020-2021 growing season a trail for screening different rye genotypes for their adult plant partial resistance to *P. recondita* f. sp. *secalis* was carried out at the Development Research Station for Plant Culture on Sands Dabuleni, located in Southern Oltenia, Romania (43°48′04″N 24°05′31″E), on sandy soil, poorly supplied with nitrogen (between 0.04-0.06%), well supplied with phosphorus (between 54 ppm and 77 ppm), reduced to a medium supplied with potassium (between 64 ppm and 83 ppm), low in organic carbon (between 0.12 and 0.48%) and weakly acidic pH to neutral (between 5.6 and 6.93).

Technological measures applied included broadcasting the fertilizers at sowing time with $N_{80}P_{80}K_{80}$, one side nitrogen fertilization during vegetation with N_{70}, starter irrigation with 250 m^3 water/ha and supplemental irrigation with 300 m^3 water/ha at heading stage. Also, weeds control was done using Dicopur Top 464 SL (1 l/ha) applied in postemergence to control annual and perennial dicotyledons accordingly with the recommendations (cereals to the formation of the first internode and the weed species in the small phase of about 2-4 leaves and a maximum of 10-15 cm high for perennial weeds).

A plant-pathogen interaction profile was observed on four selected rye genotypes (Serafino, Bintto, Inspector and Suceveana), assessed for their response to natural infection with *P. recondita* f. sp. *secalis* (Prs) (Roberge ex. Desmaz) in a randomized complete block design (RCBD) with three replications. Each plot had 5 m^2, a space of 1 m between blocks and 0.5 m between plots.

Disease observations were recorded since the first appearance of leaf rust infection on the susceptible rye genotypes until rust symptoms were fully developed (nearly at the early dough stage). All rye genotypes were phenotyped for their adult plant partial resistance using epidemiological parameters as final rust severity (FRS), area under the disease progress curve (AUDPC), relative area under the disease progress curve (rAUDPC), coefficient of infection (CI) and infection rate (IR).

Identification of the fungus *P. recondita* f. sp. *secalis* (Prs) (Roberge ex. Desmaz) and its characteristics were done in the Phytopatology Laboratory of Agriculture Faculty in University of Craiova, using MOTIC microscope. The diameter of uredinia can reach even 1.5 mm, their colour is orange to brown and their shape is round to ovoid. The average size of uredospores release from uredinia is 20 mm in diameter and colour - orange-brown. Uredospores have up to eight germ pores scattered in dense walls. Leaf rust pustules are small, circular to oval shape, with orange to light brown dusty spores (uredospores) on upper surface of leaves surrounded by a light-coloured halo (Figure 1).

Figure 1. Uredospores pulstules on rye leaves in 2021 year (original photo: Paraschivu Mirela)

Rye genotypes response was expressed in five infection types for cereals leaf rust according to Johnston and Browder (1966) (Table 1).

Table 1. Infection types of cereals leaf rust used in disease assessment at seedling stage adopted by Johnston and Browder (1966)

Infection type	Host response	Symptoms
0	Immune	No uredia or other macroscopic sign of infection
0	Nearly Immune	No uredia, but hypersensitive necrotic or chlorotic flecks present
1	Very resistant	Small uredia surrounded by necrosis
2	Moderately resistant	Small to medium uredia surrounded by chlorosis or necrosis
3	Moderately susceptible	Medium-sized uredia that may be associated with chlorosis
4	Very susceptible	Large uredia without chlorosis or necrosis
X	Heterogenous	Random distribution of variable-sized uredia on single leaf

Leaf rust severity (%) was recorded for each genotype from the time of rust first pustules appearance (booting stage) until the early dough stage (Zadoks scale) (Zadoks et al., 1974), assessing 10 tillers randomly selected and pretagged plants of the central four rows of each plot and the mean of the ten plants was considered as the value for a plot.

Rust severity was determined by visual observation and expressed as percentage coverage of leaves with rust pustules (from 1% to 100%) following Cobb's scale modified by Peterson (Peterson et al., 1948) (Table 2).

Table 2. Leaf rust severity expressed as percentage coverage of leaves with rust pustules - Cobb`s scale modified by Peterson (Peterson et al., 1948)

Category	Percentage leaf rust infection relative to susceptible check	Type of resistance
1	80-100%	Susceptible
2	50-70%	Race-nonspecific, low resistance
3	30-50%	Race-nonspecific, moderate resistance
4	10-20%	Race-specific, high resistance
5	less than 10%	Race-specific, high resistance
6	less than 5%	Effective, race-specific resistance

Final rust severity values were used to calculate Area under Disease Progress Curve (AUDPC), which shows the evolution and disease quantity on each rye genotype included in the trail, following the formula (Campbell and Madden, 1990):

$$AUDPC = \sum_{i=1}^{a}\left[\left\{\frac{Yi + Y(i+1)}{2}\right\}x(t(i+1) - ti)\right]$$

where, Yi = disease severity (%) at each measurement; ti = time in days of each measurement; a = number of Leaf Rust assessments.

Relative Area Under the Disease Progress Curve (rAUDPC) was calculated using the following formula:

rAUDPC = [AUDPC check/AUDPC assessed genotype] × 100

Average coefficient of infection (CI) was calculated by multiplying the percentage of disease severity and the constant value assigned to each infection type (Saari and Wilcoxson, 1974; Pathan and Park, 2006). The constant values were considered as R = 0.2, R-MR = 0.3, MR = 0.4, MS = 0.8 and S = 1.

Apparent infection rate (IR) as a function of time was also calculated from the two disease severity observations as a severity of leaf rust infection at the time of rust pustules appearance and every fifteen days thereafter. It was estimated using the following formula adopted by Van der Plank (1963).

Inf-rate (IR) = 1/t (ln x/1-x)

Where x = the percent of disease severity divided by 100; t = time measured in days. The apparent infection rate is the regression coefficient of ln x/1-x on t.

In order to characterize the evolution of climatic parameters (air temperature, rainfall, humidity, wind speed) into the experimental field it was used an automatic weather station (AWS).

Means were compared with the susceptible genotype Suceveana (control). The results were statistically analysed and interpreted using the analyse of variance and mathematical functions of MS Office Excel 2010 facilities.

RESULTS AND DISCUSSIONS

Globally, marginal lands make up about 21 percent (2.74 billion ha) of the total land (13.5 billion ha) area. However, about 1 558 million ha of these lands are used for agriculture, out of which about 224 to 300 million ha is classified as agriculturally marginal areas (Ahmadzai et al., 2022). The term "agriculturally marginal areas" (AMAs) refers to less-favourable agricultural areas (LFAAs) that are characterized by resource degradation, constrained agricultural potential, and low productivity of agricultural resources due to biophysical constraints like rocky terrain, harsh weather, poor soil quality, salinization, drought and erratic rainfall, among other factors that pose significant obstacles for intensive agriculture.

The challenges to achieve sustainable food security in dry areas meet the ones generated by the effects of climate change and climate variability on crops health, especially in vulnerable crop systems like cereals, associated by many authors with changes in pathogens life cycles, increased incidence, pathogenicity, genetically recombination and aggressiveness traits (Chakraborty and Newton, 2011; West et al., 2012; Chakraborty, 2013; Elad and Pertot, 2014; Fones at al., 2020; Wolfe and Ceccarelli, 2020).

The 2020-2021 cropping season was favourable to rye Leaf rust disease in the dry area in Southern Oltenia, Romania. According to Meidaner et al. (2011), *Puccinia recondita* f. sp. *secalis* (Prs) is prolific across all rye growing regions in Europe. To date only little research was done in Europe to trigger resistance breeding. Host resistance represents a sustainable and more environmentally conscious alternative to chemical control strategies (Nelson et al., 2018). Currently, there are no newly developed rye cultivars registered that are known to carry stem rust resistances. Previously, some authors reported resistant rye plants in populations from Italy, China, Sweden, Uruguay, the Czech Republic, Azerbaijan, former Yugoslavia, Lithuania, Ukraine, Bulgaria, Portugal, Finland, Great Britain and in in South African fodder rye (Solodukhina and Kobylyansky, 2001; Boshoff et al., 2019).

To predict the Leaf rust disease development, rainfalls and temperatures were taken into account.

Humidity was determined by the amount of rain of 406.00 mm, comparatively with multiannual average rainfall of 376.85 mm, while the monthly average temperature was 13.7ºC comparatively with multiannual average temperature of 12.7ºC (Figure 2).

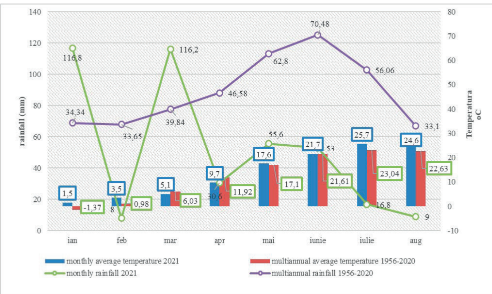

Figure 2. Climatic conditions during the study period (2021 year)

This temperature increase follows the global trend in planet warming. Thus, accordingly with a report of National Oceanic and Atmospheric Administration (NOAA, September 2020) monthly average temperature for January to August 2020 increased up to +1.03ºC (+15.03ºC) at the global level comparatively with average temperature recorded on Earth in the 20th century (+14ºC).

Rainfall amount for evaluated period was higher with 29.15 mm than multiannual amount for dry areas in Southern Romania, which led to symptoms exhibition on rye genotypes leaves in middle April 2021.

Starting with May 2021 the humidity decreased, while temperatures increased. The disease can result in significant yield losses over wide areas when the summers are warm and dry. Optimal environmental conditions for disease development are temperatures ranging from 15°C to 20°C, but the fungus can develop at the temperature of 2-35°C. The fungus needs approximately six hours of moisture on leaves to start developing. With much moisture and suitable temperatures, lesions are formed within 7-10 days and spore production reduplicate another uredospore generation (Kolmer, 2013).

Săvulescu (1953) showed that uredospores of leaf rust were visible on rye leaves at the end of May or the beginning of June, but the currently results show that in the context of climate change, with higher monthly average temperature and ununiform rainfalls, these fruiting bodies of the pathogen (uredinia with uredospores) appear earlier. These findings suggest a modification of life cycle of the pathogen *P. recondita* f. sp. *secalis* by many generation numbers and higher resistance of uredospores to increased temperature. Also, Harvell et al. (2002) suggested that rising temperatures will (i) increase pathogen development transmission, and generation number; (ii) increase overwinter survival and reduce growth restrictions during this period and (iii) alter host susceptibility.

P. recondita f. sp. *secalis* spores are spread by splashing water and wind leading to many successive infections. Meidaner (2012) showed that minimum wind speed for uredospores splashing is 2 m/s. In the experimental field the wind speed ranged between 1.5-37 km/h. There can be thousands of spores in each pustule. In case of severe attack leaf rust pustules may extend also on the leaf sheaths, stalks and husks. Symptoms vary on the degree of cultivar resistance. There are cultivars that are very susceptible and have large uredinia without generating necrosis or chlorosis in the plant tissues. Different responses are used to identify resistant varieties, ranging from tiny spots to small- to medium-sized uredinia that may be surrounded by necrotic and chlorotic areas.

Following field screening, the response of rye genotypes to leaf rust included different variation in resistance reaction ranging from moderately resistant (Serafino, Bintto), moderately susceptible (Inspector) and very susceptible (Suceveana-control). These findings were also emphasised by partial resistance traits. Adult plant data revealed that partial resistance traits (FRS, AUDPC, rAUDPC, CI and IR) showed a discrepancy in the values within parameters and genotypes (Table 3). Previous research showed similar results on the same rye cultivars (Paraschivu et al., 2021)

Table 3. Partial resistance traits to leaf rust in adult plant of four rye genotypes

Genotype/ Type of resistance	FRS	AUDPC	rAUDPC	CI	IR
Binnto	23.11**	12.55***	444.22***	6.93***	0.0300*
Serafino	30.06*	23.25**	239.78**	12.02**	0.0429
Inspector	35.18	35.57	156.73	14.07	0.0542
Suceveana	43.50	55.75	100	43.50	0.0565

FRS = Final Rust Severity; AUDPC = Area under disease progress curve; rAUDPC = Relative area under disease progress curve; CI = Coefficient of infection; IR = Infection rate.
**Significance level at P ≤ 0.01
Suceveana = control

Thus, comparatively with Suceveana genotype (control), only Binnto possessed high level of adult plant partial resistance based on the assessed traits, during 2021-2021 cropping season. Binnto recorded the lowest Final Rust Severity (FRS) (23.11%), which corresponds with low AUDPC value (12.55) and low Infection Coefficient (IC) (6.93). The differences for all resistance traits Binnto genotypes were highly significant comparatively with the control genotype. Rye genotypes Serafino and Inspector were race-nonspecific, moderate resistance, while the control Suceveana was susceptible, race-nonspecific, low resistance.

Among all adult plant partial resistance traits was noticed a highly significant correlation (Table 4).

Table 4. Correlation coefficients (r)* for disease parameters of leaf rust on rye genotypes at DRSPCS Dabuleni during 2020-2021 cropping season

Disease parameter	FRS	AUDPC	rAUDPC	CI	IR
FRS	1	0.994***	-0.945***	0.907***	0.943***
AUDPC		1	-0.906***	0.935***	0.912***
rAUDPC			1	-0.733**	-0.986***
CI				1	0.716**
IR					1

FRS = Final Rust Severity; AUDPC = Area under disease progress curve; rAUDPC = Relative area under disease progress curve; CI = Coefficient of infection; IR = Infection rate.
* Pearsons' r_{calc} values

Negative high correlations were observed between Infection rate (IR) and rAUDPC and CI in 2020-2021 cropping season. These findings indicate that although FRS, AUDPC and CI increased, the rate of infection (IR) reduced as epidemic progressed because less healthy plant tissue was available for additional infections.

Negative relation between the disease level (AUDPC) and grain yield was found. The highest significant loss percentages were found in susceptible genotypes Suceveana and moderate resistant one Serafino. The value of determination coefficient (R^2 = 0.8505), for all rye genotypes assessed, indicated that up to 85% of variation in rye yield could be explained by AUDPC variability. It was noticed a highly significant correlation between AUDPC values and grain yield (r = -0.9222***) (Figure 3).

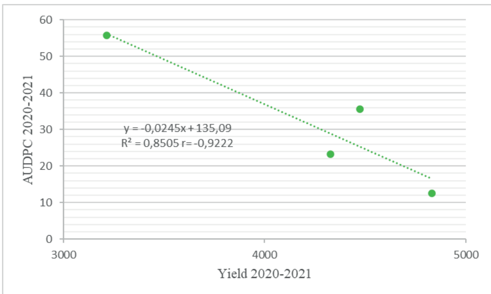

Figure 3. Relationship between Leaf rust AUDPC value and rye grain yield in 2020-2021 cropping season

Yield loses due to Leaf rust in rye in Europe were also reported previously by different authors (Solodukhina, 2002; Roux and Wehling, 2010; Meidaner et al., 2012).

The results of the experiment show that in the context of climate change the Leaf Rust is a serious disease of rye in dry marginal areas from Romania and climate variability can impact significantly the interaction between cereals and pathogens, changing host-pathogen relationship.

CONCLUSIONS

Leaf rust is a significant disease in rye worldwide causing significant yield losses. The present study was carried out to assess the adult plant response of four rye genotypes to the attack of *P. recondita* f. sp. *secalis* (Prs) (Roberge ex. Desmaz) in natural infections in dry area from Southern Romania during 2020-2021 cropping season. The response of rye genotypes to the Leaf Rust (LR) included different variation in plants reaction ranging from moderately resistant (Bintto) to moderately susceptible (Serafino, Inspector) and very susceptible (Suceveana), depending on genetic background and environmental conditions. Statistically significant differences were also observed between the control genotype (Suceaveana) and other assessed genotypes. The highest leaf rust severity was observed Suceaveana, while the lowest in Binnto. The values of Area under Disease Progress Curve (AUDPC) ranged from 12.55 (Binnto) to 55.75 (Suceveana). Binnto recorded the lowest Final rust severity (FRS) (23.11%), which corresponds with low AUDPC value (12.55) and low Infection Coefficient (IC) (6.93). The Pearsons' r_{calc} values indicated a highly significant correlation among all adult plant partial resistance traits. Also, it was noticed a highly significant correlation between AUDPC values and grain yield ($r = -0.9222^{***}$). However, under natural infection, selection on a phenotypic basis frequently results in the selection of plants that test falsely positive and does not produce adequate results. Thus, using artificial inoculation can increase selection efficiency. Therefore, investigations under both natural and artificial infections are required in order to achieve a better selection of resistant cultivars and to have a better understanding of how pathogen attacks affect grain yield.

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WHEAT YIELD AND QUALITY UNDER THE INFLUENCE OF SOWING DATE, PLANT DENSITY AND VARIETY IN SOUTH OF ROMANIA

Elena PARTAL[1], Cătălin Viorel OLTENACU[2], Mirela PARASCHIVU[3], Otilia COTUNA[4, 5], Elena Laura CONTESCU[1]

[1]National Agricultural Research and Development Institute Fundulea, 1 Nicolae Titulescu Street, 915200, Fundulea, Calarasi County, Romania
[2]Research and Development Station for Fruit Tree Growing Baneasa, 4 Ion Ionescu de la Brad Blvd, District 1, Bucharest, Romania
[3]University of Craiova, Faculty of Agronomy, 13 A.I. Cuza Street, 200585, Craiova, Romania
[4]University of Life Sciences"King Mihai I" from Timisoara, Romania, 119 Calea Aradului Street, 300645, Timisoara, Romania
[5]Agricultural Research and Development Station Lovrin, 200 Principala Street, 307250, Lovrin, Timis County, Romania

Corresponding author email: oltenacu_viorel@yahoo.com

Abstract

The efficiency of the autumn wheat crop requires the application of some general technologies, but improved with innovative and specific technological sequences, depending on the evolution of the vegetation cycle and the expected production components. The researches were performed during the 2020-2022, in the experimental field of NARDI Fundulea and aimed to study the influence of agrotechnical practices on the yields and quality of wheat. The paper presents the results obtained in experiences with sowing dates and plant density, under non-irrigation condition, in the south of the country. Recording a stable and high production of autumn wheat is possible if a good sowing quality is ensured and the optimal sowing interval and plant density per m2 are observed. The variability of individual productivity of wheat plants can increase with the delay of the sowing date by up to 10-20% for the number of grains/plant and between 10-25% for the weight of grains/plant, and these lead to a decrease for production per hectare with 1000-3000 kg/ha. The variation of climatic conditions influenced negatively wheat yield and quality.

Key words: *wheat, sowing dates, plant density, variety, yield and quality, climatic conditions.*

INTRODUCTION

Wheat (*Triticum aestivum*) is one of the most important crops and ranks first in the world, cultivated on 220 million ha and producing about 781 million tons of grain (www.fao.org). In Romania, wheat is cultivated on 2.2 million ha (www.madr.ro).

The rapid and uniform emergence of wheat seeds and the evolution of all growth phases under good conditions are closely related to the availability of soil moisture, climatic conditions and applied technological links. To see the production potential of varieties, it is necessary to take into account the relationship between rainfall and yield when determining the time of sowing (Iagaru, 1998; Lupu, 2001; Popa, 2003). Previous research on the influence of sowing time and climatic conditions on the yield capacity of winter wheat has shown that other technological conditions are also important (Epplin, 1998; Tack et al., 2015).

Previous research shows the influence and importance of sowing time and plant density on the growth and evolution of the wheat crop in different climatic conditions from the point of view of precipitation and temperature (Raza et al., 2019). Sowing time is a very important technological link to maximize wheat crop yield and quality and therefore research is focused on wheat crop response to sowing at different times and other technological factors (Paraschivu et al., 2017; Abendroth et al., 2017).

For the sowing of wheat crops in good conditions, the optimal time can be different from one agricultural area to another depending on the type of soil and climatic evolution, especially soil moisture (Donatelli et al., 2012; Paraschivu et al., 2015; Partal & Paraschivu,

2020). Wheat seeds need optimal moisture and positive soil temperatures to germinate and develop in the early stages of growth (Abendroth et al., 2017), and early sowing is now widely applied by farmers to take advantage of positive temperatures (Partal, 2020). Sowing in the optimal period leads to increased yields and avoids the unjustified delay of seed germination and the growth of premises for their low quality. In the conditions of a late sowing and low temperatures in the soil, the seeds have the gestational capacity to absorb water, but they will delay the uniform germination and the growth of the roots, which leads to a defective germination and evolution of the crop (Donatelli et al., 2012; Abendroth et al., 2017). The amount and availability of water in the soil and the appropriate temperatures for the sowing period are the limiting factors that affect the optimal plant density and finally, the yield and quality of the wheat crop. The amount and distribution of precipitation, soil water and plant density interact and directly influence the period of active crop growth both in autumn and spring (Lobell and Burke, 2008; Raza et al., 2019). Production and quality register variations between varieties that are determined by the genetic characteristics of the variety, the supply of soil with nutrients, environmental conditions, especially soil moisture and temperatures, and the technological links applied to the culture (Theago et al., 2014; Partal, 2020).
Thus, in this paper we present the results recorded in the last three years on the influence of sowing time, plant density, variety and climatic conditions on wheat yield and quality.

MATERIALS AND METHODS

The field tests were established in the period 2020-2021-2022, on a specific soil for southern Romania (cambic chernozem). Regarding the physical characteristics of the soil, the humus content is higher in the first 15 cm due to the former bedding and gradually decreases to depth.
The soil consists of several horizons:
- Ap+Aph - 0-30 cm, clay-clay-dust with 36.5% clay and permeability 492, pH 5.9.
- Am - 30-45 cm, clay-clay with 37.3% clay, compacted, DA 1.41 g/cm³, pH 5.9.
- A/B (45-62 cm), Bv1 (62-80 cm), Bv2 (82-112 cm), Cnk1 (149-170 cm), Cnk2 (170-200 cm).

Depending on the agricultural year, the water supply of the soil is favorable for field crops, groundwater at 10-12 meters.
The experimental material included two wheat variety (Izvor and Pitar), developed at the National Institute for Agricultural Research and Development in Fundulea. Variety were sown at five different dates (SD I - September 20, SD II - October 01, SD III - October 10, SD IV - October 25 and SD V - November 10) and at three plant density (500 and 600 seeds/m^2). The main plots are 240 m^2 (30 m x 8 m) and the sub-plots 48 m^2 (6 m x 8 m). The cultivation of wheat followed the maize, in the rotation of 4 years.
In terms of quality, samples were taken from each repetition and variant and determined:
- the weight of one thousand grains - WTG was weight with the Kern precision electronic scale.
- the hectolitre weight - HW- was determined with the special cylinder, followed by weighing on the Kern scale.

Using the electronic device INFRATEC 1241 Grain Analyzer, the elements of seed analysis were determined: Protein %.
The analyzes and data obtained were processed and statistically interpreted according to the analysis of variance method.

RESULTS AND DISCUSSIONS

Climatic aspects

The climatic aspects recorded during the research period showed significant differences from one year to another due to the variation in temperature and distribution of precipitation.
The agricultural year 2019/2020 was a dry one, with a high water shortage and high temperatures, compared to the multiannual average. The months with the lowest precipitation were September 2019 by 6.2 mm, compared to the multiannual average of 50.9 mm, April by 14 mm compared to 45.1 mm on average, august by 5.4 mm compared to 49.7 mm on average and July by 34.2 mm compared to 71.1 mm on average. In May and June there were almost normal rainfall amounts, 57.8 mm and 68.4 mm, respectively. The rainfall deficit affected the installation and

development of crop plants in the early phases after sowing, which had a negative impact on the final production. Temperatures higher than the annual average have exacerbated drought.

The average temperatures recorded in the 2019/2020 agricultural year were 13.5ºC, compared to the multi-annual average of 10.8ºC, with an increase of 2.7ºC.

In 2020/2021, a normal year in terms of water quantities recorded, but with an uneven distribution, especially in March, May and June (135 mm against 74.9 mm multi-year average). The temperatures registered a difference of 1.2ºC compared to the multiannual average.

In 2021/2022, the months with the lowest rainfall were September with 4.0 mm against the multi-year average of 48.5 mm, January with 4.8 mm against the multi-year average of 34.1 mm and August with 14.4 mm against the multi-year average of 49.7 mm. The highest amount of precipitation was in April with 47.6 mm, 2.5 mm above the multi-year average.

Regarding the thermal regime, in the period from October 2021 to July 2022, the values show that the monthly averages were higher than multiannual average. The temperatures registered a difference of 1.8ºC compared to the multiannual average.

In order to establish the influence of the climatic elements, on the evolution of the wheat culture, the values obtained in different phenological phases of the plants with the final production were analyzed and corroborated, in terms of quantity and quality (Table 1).

The annual climate data were compared with the multiannual average over the last 50 years, which recorded precipitation values of 584.3 mm and temperature of 10.8°C.

Table 1. The meteorological parameters in the experimental period (NARDI Fundulea, 2019-2022)

Years/Months		Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	July	Aug	Total/ Average
Precipitations (mm)	’19/’20	6.2	38.2	33.2	16.2	2.0	16.6	29.8	14.0	57.8	68.4	34.2	5.4	322.0
	’20/’21	68.6	24.0	20.0	77.6	77.0	16.2	59.0	31.0	57.6	135	21.2	24.2	611.4
	’21/’22	4.0	56.4	33.8	37.6	4.8	5.4	12.3	47.6	30.1	59.6	29.2	14.4	335.2
50 years average		**48.5**	**42.3**	**42.0**	**43.7**	**35.1**	**32.0**	**37.4**	**45.1**	**62.5**	**74.9**	**71.1**	**49.7**	**584.3**
Temperatures (ºC)	’19/’20	19.3	12.0	11.0	4.0	0.9	5.2	8.3	12.4	16.8	21.8	25.1	25.5	13.5
	’20/’21	20.8	12.8	6.2	4.0	1.6	3.2	5.1	9.7	17.2	21.1	25.3	24.2	12.6
	’21/’22	17.3	10.2	7.7	2.6	2.1	4.7	4.4	12.1	17.9	22.6	25.0	25.6	12.6
50 years average		**17.3**	**11.3**	**5.4**	**0.1**	**-2.4**	**-0.4**	**4.9**	**11.3**	**17.0**	**20.8**	**22.7**	**22.3**	**10.8**

Production and quality

Production registered differences under the influence of applied technological links. In 2020, the control variant with the sowing date of September 20 achieved a production of 3500 kg, thus becoming the lowest production in the series of factor ratings. The variant with the sowing date of October 10 recorded a production of 4500 kg/ha with 1000 kg (or 28.6%) above the values of the control variant, thus becoming the highest production in the series of factor graduations. The variant with the sowing date of October 25 had a production of 4450 kg/ha, 27.1% above the value of control variant with 3500 kg/ha.

The density at sowing showed a significant variation, so the control variant with 500 g.s./m^2 recorded a production of 4000 kg/ha.

The application of a density of 600 g.s./m^2 (g.s. - germinating seeds) led to a production of 4350 kg/ha, 8.8% more than the control, becoming the best variant in the series of graduates.

The production data after the gradations of factor C - the variety, show us that in the control version, with the Izvor variety, 4020 kg/ha were obtained, and in the version with Pitar 4120 kg/ha., 2.5% more (Table 2).

The hectoliter weight (HW) registered different values depending on the graduations of the studied factors. The highest values were recorded for the variants with sowing dates of October 25 and October 10, with 79.5 and 79.1 kg/hl, respectively.

The density at sowing led to obtaining a maximum value of 79.4 kg/hl for the variant with the density of 600 g.s./m^2, and the variant with 500 g.s./m^2 which registered 78.0 kg/hl.

The Pitar variety obtained the highest HW value, namely 79.4 kg/hl.

Weight of one thousand grains (WTG) recorded values between 44.0-45.7 g depending on the factors. The density at sowing influenced WTG in a small percentage, so that at the density of 500 g.s./m^2, 45.0 g was recorded, and at the density of 600 g.s./m^2, 45.2 g was recorded. The Pitar variety had the highest WTG value of 45.7 g, followed by the Izvor variety with an average recorded value of 45.2 g (Table 2).

Table 2. Production results obtained for wheat crop in 2020

Specification variant	Production/Diference			HW		WTG	
	(kg/ha)	(%)	Semnific.	kg/hl	%	g	%
A. Sowing time							
A1 - Mt	3500	100.0	0	77.0	100.0	44.0	100.0
A2	4050	115.7	550*	78.1	101.4**	45.1	102.5**
A3	4500	128.6	1000*	79.1	102.7**	45.7	103.8***
A4	4450	127.1	950*	79.5	103.2**	45.5	103.4***
A5	4000	114.3	500	78.4	101.8**	45.1	102.5**
DL (kg/ha/kg/hl/g)	DL = (P 5% = 550/P 1% = 1005/P 0.1% = 1910)			DL = (0.74/1.24/2.30)		DL = (1.11/1.73/3.31)	
B. Density							
B1 - Mt	4000	100.0	0	78.0	100.0	45.0	100.0
B3	4350	108.8	350	79.4	101.8*	45.2	100.4
DL (kg/ha)	DL = (P 5% = 620 /P 1% = 1075 /P 0.1% = 1980)			DL = (0.70/1.11/2.23)		DL = (1.04/ 2.11/3.35)	
C. Variety							
C1 - Mt	4020	100.0	0	78.0	100.0	45.2	100.0
C2	4120	102.5	100	79.4	101.8**	45.7	101.1*
DL (kg/ha)	P 5% = (605/P 1% = 1011 /P 0.1% = 1820)			DL = (0.73/1.19/2.31)		DL = (1.09/2.15/3.28)	

In 2021, the control variant with the sowing date of September 20 achieved a production of 3100 kg, thus becoming the lowest production in the series of factor gradations. The variant with the sowing date of October 10 recorded a production of 4660 kg/ha with 1560 kg (or 50.3%) above the values of the control variant, thus becoming the highest production in the series of factor gradations. The variant with the October 25 sowing date had a production of 4550 kg/ha, 46.8% above the control value. The density at sowing recorded significant variations, so the control variant with 500 g.s./m^2 recorded a production of 4060 kg/ha. The application of a density of 600 g.s./m^2 led to a production of 4344 kg/ha, 6.9% more than the control, becoming the best variant in the series of graduates.

The production data depending on the gradations of factor C - the variety, show us that in the control version, with the Izvor variety, 3550 kg/ha were obtained, and in the version with Pitar 3900 kg/ha., with 9.9% more (Table 3).

The hectoliter weight recorded the highest for the variants with sowing dates on October 25 and October 10, both with 78.2 kg/hl.

The density at sowing led to obtaining a maximum value of 78.6 kg/hl for the variant with the density of 600 g.s./m^2, compared to the variant with 500 g.s./m^2 which recorded 77.0 kg/hl.

Pitar variety obtained the highest weight value in hectoliter, namely 78.3 kg/hl.

The weight of one thousand grains (WTG) recorded values between 43.0-44.6 g depending on the factors.

The density at sowing influenced WTG in a small percentage, so that at the density of 500 g.s./m^2, 44.0 g was recorded and for the density of 600 g.s./m^2, 44.3 g was recorded.

The Pitar variety had the highest WTG value of 44.6 g and the Izvor variety recorded 44.1 g. (Table 3).

Table 3. Production results obtained for wheat crop in 2021

Specification variant	Production /Diference			HW		WTG	
	(kg/ha)	(%)	Semnific.	kg/hl	%	g	%
A. Sowing time							
A1 - Mt	3100	100.0	0	77.0	100.0	43.0	100.0
A2	4088	131.8	988*	78.0	101.3**	44.5	103.5***
A3	4660	150.3	1560**	78.2	101.5**	44.6	103.7***
A4	4550	146.8	1450**	78.2	101.5**	44.5	103.5***
A5	4000	129.0	900*	78.0	101.3**	44.5	103.5***
DL (kg/ha/kg/hl/g)	DL = (P 5% = 600/P 1% = 1080/P 0.1% = 1872)			DL = (0.70/1.16/2.10)		DL = (1.03/1.66/3.12)	
B. Density							
B1 - Mt	4060	100.0	0	77.0	100.0	44.0	100.0
B2	4344	106.9	284	78.6	102.1	44.3	100.7
DL (kg/ha)	DL = (P 5% = 614/P 1% = 1022/P 0.1% = 1912)			DL = (0.68/1.09/2.20)		DL = (1.03/2.08/3.31)	
C. Variety							
C1 - Mt	3550	100.0	0	77.0	100.0	44.1	100.0
C2	3900	109.9	350	78.3	101.7**	44.6	101.1*
DL (kg/ha)	P 5% = (591 /P 1% = 1081,1 /P 0,1% = 1799,0)			DL = (0.72 / 1.13 / 2,28)		DL = (1.09/2.11/3.22)	

In 2022, the control variant with the sowing date of September 20 achieved a production of 3100 kg, thus becoming the lowest production in the series of factor gradations (Table 4).
The variant with the sowing date of October 10 recorded a production of 3980 kg/ha with 880 kg (or 28.4%) above the values of the control variant, thus becoming the highest production in the series of factor gradations. The variant with the October 25 sowing date had a production of 3900 kg/ha, 25.8% above the control value.

The density at sowing recorded significant variations, so the control variant with 500 g.s./m^2 recorded a production of 2600 kg/ha. The application of a density of 600 g.s./m^2 led to a production of 3410 kg/ha, 33.4% more than the control, becoming the best variant in the series of graduates.
The production data after the gradations of factor C - the variety, show us that in the control version, with the Izvor variety, 3500 kg/ha were obtained, and in the version with Pitar 3750 kg/ha, with 7.1% more (Table 4).

Table 4. Production results obtained for wheat crop in 2022

Specification variant	Production /Diference			HW		WTG	
	(kg/ha)	(%)	Semnific.	kg/hl	%	g	%
A. Sowing time							
A1 - Mt	3100	100.0	0	75.1	100.0	42.0	100.0
A2	3700	119.4	600*	76.0	101.2*	43.4	103.3***
A3	3980	128.4	880*	76.7	102.1***	43.2	102.8**
A4	3900	125.8	800*	76.7	102.1***	43.4	103.3***
A5	3800	122.6	700*	76.5	101.9**	43.2	102.8**
DL (kg/ha/kg/hl/g)	DL = (P 5%= 480/P 1% = 890/P 0.1% = 1560)			DL = (0.53/1.06/2.00)		DL = (1.03/1.51/3.06)	
B. Density							
B1 - Mt	2600	100.0	0	75.1	100.0	43.1	100.0
B2	3410	133.4	810**	76.5	101.9**	43.6	101.2*
DL (kg/ha)	DL = (P 5% = 440 /P 1% = 802 /P 0.1% = 1590)			DL = (0.61/1.02/2.13)		DL = (1.00/2.12/3.19)	
C. Variety							
C1 - Mt	3500	100.0	0	75.2	100.0	43.2	100.0
C2	3750	107.1	250	76.4	101.6**	43.6	100.9
DL (kg/ha)	P 5% = (480/P 1% = 960/P 0.1% = 1711)			DL = (0.70/1.12/2.25)		DL = (1.01/2.10/3.05)	

The hectoliter weight recorded the highest for the variants with sowing dates on October 25 and October 10, both with 76.7 kg/hl.
The density at sowing led to obtaining a maximum value of 76.5 kg/hl for the variant with the density of 600 g.s./m^2, compared to the variant with 500 g.s./m^2 which recorded 75.1 kg/hl.
The Pitar variety obtained the highest value of hectoliter weight, namely 76.4 kg/hl.
The weight of one thousand grains (WTG) recorded values between 42.0-43.4 g depending on the factors. The Pitar variety had the highest WTG value of 43.6 g.

The protein content recorded the highest values in 2021, these being the associated variant sown on October 25 with a density of 500 g.s./m^2 for the Pitar variety (with 13.8%) followed by the associated variant sown on October 1 with a density of 600 g.s./m^2 for the Izvor variety (with 13.5%) (Figure 1).
Among the existing functions in the Windows program - linear, logarithmic, polynomial, power and exponential - the polynomial function has the highest regression coefficient for the connection between agrotechnical measures (sowing date/seeds density/variety) and protein content in wheat (Figure 1).

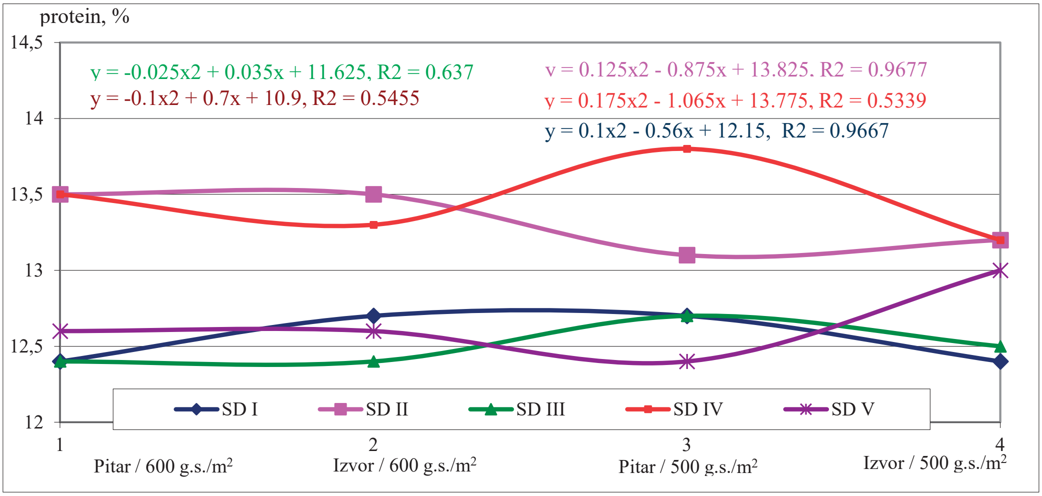

Figure 1. Correlation between agronomic measures and wheat protein content

Increasing the possibilities of choosing the sowing date and establishing the sowing density and especially the association of these factors ensure the correlations as very positive, with regression coefficients between 0.54 and 0.96. Early sowing, associated with a high sowing density, resulted in a reduced amount of protein, and the regression coefficient is 0.54.

CONCLUSIONS

The results of the study and the analyzes presented in this paper showed that sowing date, plant density and variety interact with water supply and affect the quantity and quality of wheat production. The technological links applied to the wheat crop have contributed sustainably to the growth and stability of crops and their quality.

Taking into account the technology applied to wheat, the following technological links were noted: the sowing time should be between October 10 and October 25, even delayed compared to the optimal time recommended until now; density at sowing of 600 g.s./m^2; the Pitar variety which ensures a stable and high quality production followed by the Izvor variety.
Temperatures and non-uniform distribution of precipitation affect wheat plants in any phase of the vegetation, with a negative impact on the final production and its quality.
The phenomenon of drought influences by intensity and duration, sometimes to the point of compromising the culture and in this case the drought resistance of the variety has a decisive role.

The protein content of wheat groups the variants as follows: very good quality (protein > 13%), good (12-13%) and satisfactory (8-12%). Thus, sowing in the optimal period or later and densities of 600 g.s./m^2, resulted in an increase in the protein content to values between 12-13.8%, compared to the control variant.

In the years 2020 and 2022, the drought affected the final productions regardless of the technological links applied.

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THE INFLUENCE OF FERTILIZATION ON THE YIELD AND QUALITY OF SOME ROMANIAN WINTER WHEAT VARIETIES UNDER THE CONDITIONS OF CENTRAL MOLDOVA

Andreea-Sabina PINTILIE, Andreea ENEA, Alexandra LEONTE, Paula Lucelia PINTILIE, Roxana Georgiana AMARGHIOALEI, Lorena Diana POPA

Agriculture Research-Development Secuieni, 377 Principala Street, Secuieni, Neamt, Romania

Corresponding author email: andreeasabina97@yahoo.com

Abstract

Breeding research for the common wheat crop has focused on quality and yield. These are closely related to each other because high yield without adequate quality or good quality without satisfactory production are not profitable for the farmer. At A.R.D.S. Secuieni, in the period 2019-2022, Romanian winter wheat varieties were tested in order to establish their yield and quality. These were influenced by the variety, the climatic conditions but also by the phase fertilization applied. On average in the three years studied, the productions had values between 7108 kg/ha (Pitar) and 8442 kg/ha (Semnal) in the case of fertilized variants and between 6201 kg/ha (Miranda) and 7669 kg/ha (Abundent) in the case of unfertilized variants. Regarding the quality of common wheat varieties, the content in protein, starch and gluten was monitored.

Key words: *quality, wheat, yield.*

INTRODUCTION

Common wheat (*Triticum aestivum* ssp. *vulgare* L.) is the main source of flour, used to bake bread, being cultivated on extensive areas in all areas of the globe due to its yield and quality clearly superior to other cereals. The bran obtained from the processing of wheat grains constitute a good fodder for animals (Bîlteanu et al., 1979).

Wheat grains are rich in protein and gluten, and the breeding program is aimed at increasing the content in these indices (Șerban et al., 2022).

In Romania, two of the centres dealing with the improvement of common wheat are N.A.R.D.I. Fundulea and A.R.D.S. Turda, and their creations are the subject of research carried out during 2019/2022 at A.R.D.S. Secuieni and presented in this paper.

The specialized literature describes the factors that determine the wheat quality, as being genetics, applied crop technology and environmental conditions. This factors are decisive in the growth and development of wheat. The protein accumulation is determined by higher temperatures during flowering while lower temperatures, determines a longer period vegetative that influences the starch accumulation in larger quantities (Mogârzan, 2012; Roman et al., 2011).

The breeders, considering the growing demand for raw material for baking purposes, focused on increasing the wheat varieties yield but also on quality, these being closely related to each other (Marinciu et al., 2022).

This paper presents data on the yield results of some wheat varieties experienced in two comparative crops systems: fertilized and unfertilized.

MATERIALS AND METHODS

The A.R.D.S. Secuieni is located in the SE part of Neamt county, between the geographical coordinates of 26°5' east longitude and 46°5' north latitude, at an altitude of 205.7 m above sea level. The headquarters of the unit is located in the Secuieni commune, at a distance of approximately 10 km from the municipality of Roman and 30 km from the municipality of Bacau (https://www.scda.ro/).

In order to establish the influence of fertilization on the yield and quality of the winter wheat, two monofactorial experiments were placed: an unfertilized and a fertilized one with NPK 18:46:0 in autumn and ammonium nitrate (NH_4NO_3) in the spring. The doses applied in the autumn of NPK were 36 kg N/ha and 92 kg P_2O_5/ha and in the spring were administered 69 kg N/ha.

The behavior of 10 varieties of Romanian winter wheat were followed and determinations were made regarding the number of grains per ear, weight of grains per ear, weight of one thousand grains, hectoliter weight, yield, protein, gluten and starch content.

The method of placing experiments - randomized blocks in 3 repetitions. The experimental plots were 10 square meters (10 meters long and 1 meter wide). After elimination, the harvestable surface remained 8 square meters.

During the vegetation period no chemicals were used to combat diseases and pests.

Regarding the thermal regime recorded at A.R.D.S. Secuieni in the period 2019-2002, an increase in temperatures is observed due to the decrease in precipitation.

In the three years studied, the annual average temperatures recorded were higher than the multiannual average.

On average, temperatures have increased in the last three years by 1.2°C compared to the multiannual average (Table 1).

Table 1. Temperatures recorded at A.R.D.S. Secuieni between 2019-2022 and comparison with the multiannual average

Month	Agricultural year			Multiannual average	Average 2019/2022	Deviation
	2019/2020	2020/2021	2021/2022			
X	10.8	12.7	8.0	9.1	10.2	1.1
XI	7.8	3.9	5.6	3.5	5.2	1.7
XII	1.8	1.7	-0.2	-1.7	0.4	2.1
I	-0.6	-0.7	-0.1	-3.9	-1.3	2.6
II	3.4	-0.4	2.6	-2.2	0.9	3.1
III	6.2	2.9	2.7	2.8	3.7	0.9
IV	10.0	7.5	9.5	9.5	9.1	-0.4
V	13.9	14.7	16.3	15.4	15.1	-0.3
VI	20.0	19.2	20.7	18.8	19.7	0.9
VII	20.9	22.2	22.2	20.4	21.4	1.0
VII	22.2	20.5	22.7	19.5	21.2	1.7
IX	18.0	14.4	14.7	15.0	15.7	0.7
Average	11.2	9.9	10.4	8.9	10.1	1.2

The pluviometric regime between 2019-2022 recorded lower values compared to the multiannual average.

The driest year was 2021-2022, with a deviation from the multiannual average of 283.5 mm (Table 2).

On average, the driest months were July and April with deviations from the multiannual average of -25.8 mm and -37.0 mm (Table 2).

Table 2. The precipitation recorded at A.R.D.S. Secuieni between 2019-2022 and the comparison with the multiannual average

Month	Agricultural year			Multiannual average	Average 2019/2022	Deviation
	2019/2020	2020/2021	2021/2022			
X	33.0	27.2	3.0	38.2	21.1	-17.1
XI	14.6	7.4	10.8	28.4	10.9	-17.5
XII	6.2	38.2	39.0	25.4	27.8	2.4
I	2.0	12.2	5.4	20.1	6.5	-13.6
II	16.0	10.8	4.6	19.5	10.5	-9.0
III	10.2	31.8	0.8	26.9	14.3	-12.6
IV	1.2	23.8	38.4	46.9	21.1	-25.8
V	69.6	31.4	20.8	65.7	40.6	-25.1
VI	72.6	79.4	56.6	85.0	69.5	-15.5
VII	39.0	51.6	35.2	82.3	45.3	-37.0
VIII	51.2	76.8	15.2	60.2	64.0	3.8
IX	60.4	9.2	31.0	45.7	33.53	-12.2
Average	376.0	399.8	260.8	544.3	365.2	-179.1

The data obtained were processed according to the variance analysis method (Leonte & Simioniuc, 2018).

RESULTS AND DISCUSSIONS

The two key components of wheat yield are the number of grains per ear and the individual weight of grains. The number of grains/ear is dependent on the ability to differentiate the fertile spikelets in the ear and the nutrition level that the plants have at their disposal at the time. From previous research it is known that the number of grains in the ear is a feature of the variety (Roman et al., 2011).

Analysing the number of grains/ear in the comparative wheat crops experimented in the conditions of A.R.D.S. Secuieni, it was found that on average, in the fertilized system, the highest number of grains/ear, of 36.0 was obtained for the variant sown with the Codru variety, and the smallest number of grains/ear, of 30.43 was obtained for the variant sown with the Pitar variety. In the unfertilized system, the change in the number of grains/ear was between 27.87 (Glosa) and 33.8 (Semnal).

The weight of the grains/ear had average values ranging from 1.22 g (Pitar) to 1.62 g (Signal) in the case of fertilized variants and between 1.04 g and 1.42 g in the same varieties and in the case of unfertilized variants.

The mass of a thousand grains (TGW) is one of the most important productivity indices and in the conditions of A.R.D.S. Secuieni was influenced by the fertilization applied, on average in the case of fertilized variants with higher values, between 36.23 g (Otilia) and 44.23 g (Andrada) and in the unfertilized

experience, between 36.71 g (Voinic) and 42.74 g (Andrada).
The hectolitre mass (HM) of wheat refers to the weight of a standardised volume of wheat, measured under given conditions (Mogârzan, 2012). The hectolitre mass values determined at the genotypes experienced ranged from 78.17 kg/hl (Miranda) to 81.30 kg/hl (Ursita) in the fertilized system and ranged from 76.57 kg/hl (Miranda) to 79.80 kg/hl (Otilia) in the unfertilized system (Table 3).
Most of the current varieties correspond under normal conditions the requirements of the market regarding hectolitric mass, but efforts are needed to improve it resistance to drought and heat, to prevent low values of HM in the conditions unfavorable to filling the grains (Marinciu et al., 2022).

Table 3. Average of the yiled indices in 2019-2022 recorded at A.R.D.S. Secuieni

Variety	Number of grains/ear		Diff.	Grain weight/ear		Diff.	TGW (g)		Diff.	HM (kg/hl)		Diff.
	Fert.	Unfer		Fert	Unfert.		Fert.	Unfer		Fert.	Unfer	
Glosa (Mt)	31.70	27.87	3.83	1.42	1.05	0.37	41.70	39.18	2.52	80.07	78.70	1.37
Miranda	33.37	30.00	3.37	1.41	1.05	0.36	38.90	37.64	1.26	78.17	76.57	1.60
Otilia	37.27	36.87	0.40	1.30	1.24	0.07	36.23	37.19	-0.96	80.60	79.80	0.80
Pitar	30.43	29.83	0.60	1.22	1.04	0.19	41.31	40.11	1.19	79.13	77.60	1.53
Semnal	34.90	33.80	1.10	1.62	1.42	0.20	40.57	41.76	-1.19	79.37	78.80	0.57
Ursita	32.27	30.63	1.63	1.35	1.14	0.21	38.40	37.54	0.87	81.30	79.03	2.27
Voinic	31.47	31.43	0.03	1.26	1.19	0.07	37.88	36.71	1.17	80.57	79.07	1.50
Abund	35.57	32.50	3.07	1.34	1.15	0.19	38.70	38.14	0.56	78.90	77.73	1.17
Andrada	32.57	29.27	3.30	1.48	1.14	0.34	44.23	42.74	1.49	79.47	78.67	0.80
Codru	36.00	31.30	4.70	1.59	1.29	0.31	43.86	42.61	1.25	79.20	78.30	0.90
Average	33.55	31.35	2.20	1.40	1.17	0.23	40.18	39.36	0.82	79.68	78.43	1.25

The average grain yield obtained at the fertilized system showed variations from 7108 kg/ha (Pitar) to 8442 kg/ha (Semnal). Compared to the yield obtained at control, the Glosa variety, two variants achieved yield increases interpreted as distinctly significant (Voinic and Abundant) and the Semnal variety recorded a yield increase interpreted as very significant, with an increase compared to the control of 935 kg/ha (Table 4).
The yield obtained in the unfertilized system showed variations from 6201 kg/ha (Miranda) to 7669 kg/ha (Abund). Compared to the yield obtained with the control variety, the Glosa variety, three variants exceeded the control, being statistically interpreted as distinctly significant (Semnal and Abund) and significant (Ursita) (Table 4).

Table 4. The average yields obtained for autumn wheat during 2019-2022 at A.R.D.S. Secuieni

Variety	Average production 2019/2022 (Fertilized)	Difference from the control	Signifi.	Average production 2019/2022 (Unfertilized)	Difference from the control	Signif.
Glosa (Mt)	7507	Mt	-	6683	Mt	-
Miranda	7444	-64		6201	-482	
Otilia	7800	292		7172	489	
Pitar	7108	-399	o	6391	-292	
Semnal	8442	935	***	7579	896	**
Ursita	7699	191		7312	629	*
Voinic	7955	447	**	6699	16	
Abund	8251	744	**	7669	986	**
Andrada	7565	58		6986	302	
Codru	7867	359		7019	336	
LSD 5%	396			566		
LSD 1%	563			805		
LSD0,1%	815			1165		

Analysing the quality of the grains, from data presented in Table 5 it can be noticed that there is a major difference between the quality of the wheat grains obtained in the fertilized system compared to those obtained in the unfertilized system.
The protein content of the grains varied on average from 12.68% (Miranda) to 14.62% (Pitar) in the fertilized system and from 11.45% to 12.65% in the same varieties in the unfertilized system.
The largest differentiation of the percentage of protein obtained in the two systems was observed in the variant sown with the Pitar variety, this being 1.97% (Table 5).
As with the protein content, a noticeable differentiation was observed in the case of the percentage of gluten, which was much higher in the fertilized variants. Thus, its variation was between 25.40% (Miranda) and 29.97% (Pitar) in fertilization system and between 22.57% and 25.38% in the same varieties at the unfertilized system. The differentiation between the two systems was higher in the case of gluten compared to protein, being maximum in the variant sown with the Pitar variety, of 4.58% (Table 5).
As for the starch content of wheat grains, it was higher in the case of unfertilized variants with values ranging from 68.72% (Pitar) to 70.07% (Miranda) and between 66.48% (Ursita) and 69.03% (Miranda) in the case of fertilized variants (Table 5).

Table 5. The average values of the quality indices registered between 2019-2022 at A.R.D.S. Secuieni

Variety	PROTEIN (%)		Diff.	GLUTEN (%)		Diff.	STARCH (%)		Diff.
	Fert.	Unfert.		Fert.	Unfert.		Fert.	Unfert.	
Glosa (Mt)	13.83	12.32	1.52	28.03	24.55	3.48	68.03	69.02	-0.98
Miranda	12.68	11.45	1.23	25.40	22.57	2.83	69.03	70.07	-1.03
Otilia	13.58	11.70	1.88	27.47	23.12	4.35	67.87	69.23	-1.37
Pitar	14.62	12.65	1.97	29.97	25.38	4.58	67.22	68.72	-1.50
Semnal	13.70	12.30	1.40	27.67	24.50	3.17	68.20	69.13	-0.93
Ursita	13.43	11.78	1.65	27.18	23.28	3.90	66.48	69.88	-3.40
Voinic	13.90	12.03	1.87	28.18	23.87	4.32	67.63	69.17	-1.53
Abund	13.48	11.95	1.53	27.27	23.67	3.60	67.92	69.03	-1.12
Andrada	13.30	11.63	1.67	26.80	23.02	3.78	68.18	69.67	-1.48
Codru	13.05	11.55	1.50	26.30	22.82	3.48	68.23	69.53	-1.30
Average	13.56	11.94	1.62	27.43	23.68	3.75	67.88	69.35	-1.47

CONCLUSIONS

As a result of the researches carried out on the adaptability of some genotypes of autumn wheat in the conditions of the Center of Moldova, the following conclusions were drawn:

-on average, in the comparative fertilized system, the largest number of grains/ear, of 36.0 was obtained at the variant sown with the Codru variety, and the smallest number of grains/ear, of 30.43, was obtained for the variant sown with the Pitar variety;

- in the unfertilized system, the number of grains/ear was between 27.87 (Glosa) and 33.8 (Semnal);
- the weight of the grains/ear had average values ranging from 1.22 g (Pitar) to 1.62 g (Semnal) in fertilization system and between 1.04 g and 1.42 g for the same varieties in the unfertilized system;
- the TGW in the fertilized system ranged from 36.23 g (Otilia) to 44.23 g (Andrada) and between 36.71 g (Voinic) and 42.74 g (Andrada) to the unfertilized system;
- the HM ranged from 78.17 kg/hl (Miranda) to 81.30 kg/hl (Ursita) in the fertilized system and ranged from 76.57 kg/hl (Miranda) to 79.80 kg/hl (Otilia) in the unfertilized system;
- the average of the yields obtained by the wheat varieties had values between 7108 kg/ha (Pitar) and 8442 kg/ha (Signal) in the fertilized system and between 6201 kg/ha (Miranda) and 7669 kg/ha (Abundant) in the unfertilized system;
- the starch content had higher values in the case of unfertilized variants with values between 68.72% (Pitar) and 70.07% (Miranda) and between 66.48% (Ursita) and 69.03% (Miranda) in the case of fertilized variant;
- the protein content of the grains varied on average from 12.68% (Miranda) to 14.62% (Pitar) in the fertilized system and from 11.45% to 12.65% in the same varieties in the unfertilized system;
- the gluten content varied between 25.40% (Miranda) and 29.97% (Pitar) in fertilization system and between 22.57% and 25.38% in the same varieties in the unfertilized system.

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RESEARCH ON THE ATTACK PRODUCED BY *Tanymecus dilaticollis* Gyll. (Coleoptera: Curculionidae) IN THE CONDITIONS OF CENTRAL MOLDOVA, ROMANIA

Paula Lucelia PINTILIE, Elena TROTUŞ, Roxana Georgiana AMARGHIOALEI, Alexandra LEONTE, Andreea ENEA, Andreea Sabina PINTILIE

Agricultural Research-Development Station Secuieni, 377 Principala Street, Secuieni, Neamt, Romania

Corresponding author email: andra29nt@yahoo.com

Abstract

One of the most polyphagous pests encountered in maize crops is Tanymecus dilaticollis Gyll. which, are consuming the plants foliage in the first phenophases of vegetation and reduce the crop density. Between 2018 and 2021, the series of research carried out in the Center of Moldova concerning the T. dillaticollis Gyll. pest continued and consisted in monitoring the insect attack produced at: (I) maize sown in different sowing timing and (II) different Romanian and foreign maize genotypes. The maize seeds were treated with systemic insecticide from the neonicotinoid class (imidacloprid 600 g/l) according to the disclaimers received year after year from the Ministry of Agriculture in Romania. In the experimented period, the lowest degree of attack was recorded in the late sowing time, at the beginning of May and in mid-May compared to the optimal sowing time, and the most affected by the attack of hibernating adults was the maize sown early. Regarding the attack produced in Romanian and foreign maize genotypes, it was found five of the hybrids experimented registered attack below the average experience. The results show that sowing time and maize genotype have a smaller role as opposed to spring weather conditions which are influencing the attack size and adult density in the maize crop.

Key words*: maize genotype, sowing time, maize weevil.*

INTRODUCTION

T. dilaticolis Gyll. is one of the most well-known pests of corn and sunflower crops in Romania (Georgescu, 2014). Under the conditions in Romania, the insect has one generation per year, the hibernation takes place in the adult stage in the soil at a depth of 40 cm even up to 100 cm (Paulian, 1972). Being a thermophilic insect, the weevils come out to the surface of the soil when the temperature exceeds 9°C and start feeding on weeds and continue on agricultural crops that are emerging (Georgescu, 2019). The most affected areas in Romania by the insect attack are in south, south-east and east of Romania and insect does not create economic losses in the rest of the areas (Georgescu et al., 2012; Bărbulescu, 2001; Trotus et al., 2019). The insect attack on agricultural crops is easy to recognize, the weevils consume the young leaves of the plants from their emergence until the development of 6 leaves (Rosca et al., 2011) and leads to the decrease in plant density per ha, to the delayed development of the attacked plants and increasing the production cost if it is necessary to apply additional crop protection measures when the density of weevils exceeds the EDT (5 specimens/m^2) (Dudoiu et al., 2018). What influences the growth of the weevil population and the attacks intensification is a combination of factors made up of climatic conditions and agrotechnical factors. The influence of climate change on the insect was studied by Badiu et al. (2019) who found that the increase in temperature in the second part of April influences the weevils attack and their density in agricultural crops. Winters deficient in precipitation and with higher average temperature values together with hot and dry springs lead to the shortening of the weevils hibernation, which appears earlier and develops large populations, that consume maize plants, sunflowers, other agricultural crops or weeds (Georgescu et al, 2015). Regarding agronomic factors, crop rotation by avoiding monoculture and the use of systemic insecticides in the seed chemical treatment reduce the weevils attack and spread (Georgescu et al., 2019; 2020). Being a polyphagous insect, the weevils find good feeding conditions in several agricultural crops, but the one that provides them with the best conditions for development is maize crop (Voinescu and Bărbulescu, 1998; Popov et al., 2006). The effectiveness of seed chemical treatment was studied by a number of Romanian researchers (Georgescu et al., 2012; 2014; 2015; 2016; 2019; 2020; Trotuș et al., 2011; 2017; 2019), but according to the 2013 EU directive (NO 485/2013) their use was prohibited. Year after year, Romania obtains derogation for the use of systemic insecticides in the chemical treatment of maize, sunflower and beet seeds.

In the Center of Modova, the harmful entomofauna to maize crops is abundant, but of all the species inventoried, of economic importance due to the damage it cause are the soil pests: *Agriotes* spp. and *T. dilaticollis* Gyll., that affect the crops in the period between seed germination - the plant emergence - the formation of the first 3-5 leaves (Trotuș et al., 2021). The present work presents data regarding the influence of some agronomic factors on the attack produced by *T. dilaticollis* Gyll. to maize crops.

MATERIALS AND METHODS

Research location

The research was carried out within the Agricultural Research and Development Station Secuieni - Neamț located at the geographical coordinates of 26°5' east longitude and 46°5' north latitude. A.R.D.S. Secuieni is located in the Central Plateau of Moldova, located in the eastern part of the country where the altitude drops below 250 m and is replaced by the forest-steppe area. From a practical point of view for agriculture, the relief includes extensive interfluvial plains, meadows and terraces (Trotuș et al., 2020).

The climate temperate continental (D.f.b.) with short springs, cool summers and harsh winters in Koppen (1936) climate classification.

In order to reduce the *T. dillaticollis* Gyll. attack the following experimental factors were studied:

1. Sowing time: Five sowing times were experienced from the beginning of April until mid May. The hybrid used was Turda star.

- Sowing time I - extra-early sowing (beginning of April)
- Sowing time II - early sowing (mid - April)
- Sowing time III - optimal sowing (end of April) for the conditions in the Center of Moldova
- Sowing time IV - semi-late sowing (beginning of May)
- Sowing time V - late sowing (mid - May)

2. Cultivated maize genotype: In this experience, several maize hybrids were sown in the optimal sowing time for the conditions in Central Moldova, at the end of April, and were studied: Vibrion (FAO 290), Inventive (FAO 300), Turda star (FAO 370), Turda 248 (FAO 380), Turda 344 (FAO 380), Turda 332 (FAO 380), Method (FAO 380), Kerala (FAO 400), Olt (FAO 430) and Messir (FAO 500).

The two experiments were laid out in the field according to the randomized block design with three replications. Each variant in each replicate had four rows of plants and all determinations were performed on plants from the two middle rows. The length of one variant was 10 m, and the width 2.8 m. The area of one variant was 28 square meters. Sowing was done manually, at a distance of 22 cm between the grains, ensuring a density of 65,000 b.g./ha. The seeds were treated before sowing with Nuprid 8 l/to (imidacloprid 600 g/l) according to the derogation received from year to year. An early pre-emergence herbicide Adengo 0.3 l/ha and a mechanical harrow were applied to control weeds. No vegetation treatment was applied.

The experiments were located on a typical cambic chernozem soil, with water pH 6.29, humus content 2.3, nitrogen index 2.1, mobile P_2O_5 content 39 ppm, mobile K_2O content 161 ppm.

Attack determination

The attacks determination for sowing time and genotypes was performed on the middle rows from each variant in each replicate.

The attacks determination produced by the pest was done according to the 0-6 scale, where: 0 = no attack; 1 = attack between 1 and 3%; 2 = attack between 4 and 12%; 3 = attack between 13 and 25%; 4 = attack between 26 and 75%; 5 = attack between 76% and 100%. After the marks were awarded, the frequency, intensity and degree of the attack was calculated.

The frequency of attack (F%) is the ratio between the number of attacked plants or organs of the attacked plant (n), related to the number of plants or vegetative organs observed (N).

Attack intensity (I%) represents, in fact, the percentage of attacked plants or organs of the plant destroyed by the pest.
The degree of attack (GA%) is equal to the product of these two indicators.

Statistical analyses

This paper presents the results obtained between 2018 and 2022 and includes the average values of the degree of attack of the recorded attack level. Experimental data from each experiment were calculated in Microsoft Excel. The obtained results were processed with ANOVA (analysis of variance - Fisher test) and limit differences - LSD (where p<5% = */O; p < 1% **/OO; p < 0.1% = ***/ OOO).

Weather conditions

Meteorological data were recorded at the facility's own weather station, which is a VANTAGE PRO 2 type. It is located in the experimental field, and data recording and computer storage is automated. To characterize the years from a climatic point of view, we used the data on the average air temperature (°C) at 2 m height and the amount of precipitation (mm).

RESULTS AND DISCUSSIONS

The climatic conditions during the determination period show that the spring months were atypical. In 2018, the recorded temperatures exceeded the multi-year monthly averages, and the months of April and May recorded summer temperatures and the lack of precipitation raised problems for crop emergence and contributed to the early weevils appearance (Tables 1 and 2).
In 2020, the two months were atypical in terms of average temperatures and precipitation, April being hot (10°C) and very dry (1.2 mm) followed by average temperatures dropping in May (13.9°C), and the precipitation were recorded in normal amounts (69.6 mm). One of the most atypical springs was recorded in April 2021 when the average temperature decreased by -2.1°C, and May recorded average temperatures (14.7°C) close to the multiannual average (15.4°C). On the other hand, from a pluviometric point of view, the months were very dry, the precipitation deficit being between -21.6 mm and -34.2 mm (Tables 1, and 2). In 2022, the thermal regime was normal (9.5°C) in April and normal to warm in May (16.3°C). The precipitation was reduced, the months being dry, these conditions being favorable for the emergence and attack of hibernating adults. From the data published in the specialized literature, we find that the insect is heat-loving, and the lack of precipitation contributes to the attacks increses (Popov et al., 2006), Analyzing the climatic conditions recorded in the Center of Moldova, it is found that it were favorable for the weevils appearance, spread and attack.

Table 1. The average temperatures recorded in April and May between 2018 and 2022, Secuieni - Neamț

Year	April				May			
	T air, °C	T soil, °C	Multiannual average (1962-2021)	Deviation (Characterization)	T air, °C	T soil, °C	Multiannual average (1962-2021)	Deviation (Characterization)
2018	14.3	17.1	9.6	+4.6 (very warm)	17.8	21.6	15.4	+2.4(very warm)
2020	10	12.3		1.4 (warm)	15.3	17.9		-1.5 (cool)
2021	7.5	9.3		-2.1 (very cool)	13.9	16.8		-0.7(normal)
2022	9.5	11.2		0.1 (normal)	14.7	17.2		0.9(normal to warm)

Table 2. Precipitation recorded in April and May between 2018-2022, Secuieni - Neamț

Year	April monthly rainfall ∑ (mm)	Multiannual average (1962-2021)	Deviation (Characterization)	May monthly rainfall ∑ (mm)	Multiannual average (1962-2021)	Deviation (Characterization)
2018	14.8	45.4	-30.6 (very dry)	23.4	65.6	-42.2 (very dry)
2020	1.2		-44.2 (very dry)	69.6		+4 (normal)
2021	23.8		-21.6 (very dry)	31.4		-34.2 (very dry)
2022	38.4		-8.5 (less dry)	20.8		-44.8 (very dry)

The attack produced by weevils on maize sown in different sowing times was monitored in 2018, 2020, 2021 and 2022 under the conditions of Central Moldova, Romania.
Analyzing the determinations from 2018 regarding the insect attack, it is found that high temperatures in spring positively influenced the insect appearance, the attack frequency register

100% in all five sowing times experienced. The lowest degree of attack was recorded at the optimal sowing time (IIIrd), of 13.7%, compared to the rest of the sowing times, where the increase in attack at earlier and later sown maize by 23.6% (Ist sowing time) and 28.1% (Vth sowing time) (Figure 1).

In 2020, the insect's activity was positively influenced by the temperatures in April, but the temperatures dropping in May reduced the attacks. The attack frequency recorded in the five tested sowing times was between 0.1% (Vth sowing time), and 1.3% (IIIrd sowing time) (Figure 1). Although April 2021 was cool, the spring drought and normal temperature conditions in May favored the insect's activity. The highest attacks were recorded in the first two sowing times, where weevils consumed the leaf foliage. The degree of attack was maximum in Ist and IInd sowing times, of 45.3% and 32%, and greatly diminished in sowing times IIIrd and IVth, of 25% and 11.1% (Figure 1).

These atypical conditions in the two months of 2021 determined the mass weevils appearance to primarily attack early maize that emerges (Figure 1).

In 2022, rising temperatures and lack of precipitation influenced adult emergence and attack, which were concentrated, as in previous years, on maize sown early in the I and II sowing times compared to maize sown in the optimal time. The late sowing times, IV and V, recorded reduced attacks of hibernating adults, below 2% (Figure 1). Considering that insect is active when ground temperatures exceed 18^{o}C, and average daily temperatures exceed 20^{o}C (Georgescu, 2019), in May 2018 and 2021, the insect had favorable conditions, with temperatures close to 18^{o}C recorded at ground level (Table 1). Observations show that weevils populations focus their attacks on young plants that sprout first in the spring. As other maize crops are emerging, the weevils feed on the new plants. Once maize fields have passed the BBCH 12-14 stage, the crop's vulnerable stage to weevils, attacks focus on late-sowed maize.

Our results are similar to those published by Georgescu et al. (2019) which show that changing the sowing time does not reduce the insect attack in maize and cannot replace the chemical treatment of the seed with systemic insecticides.

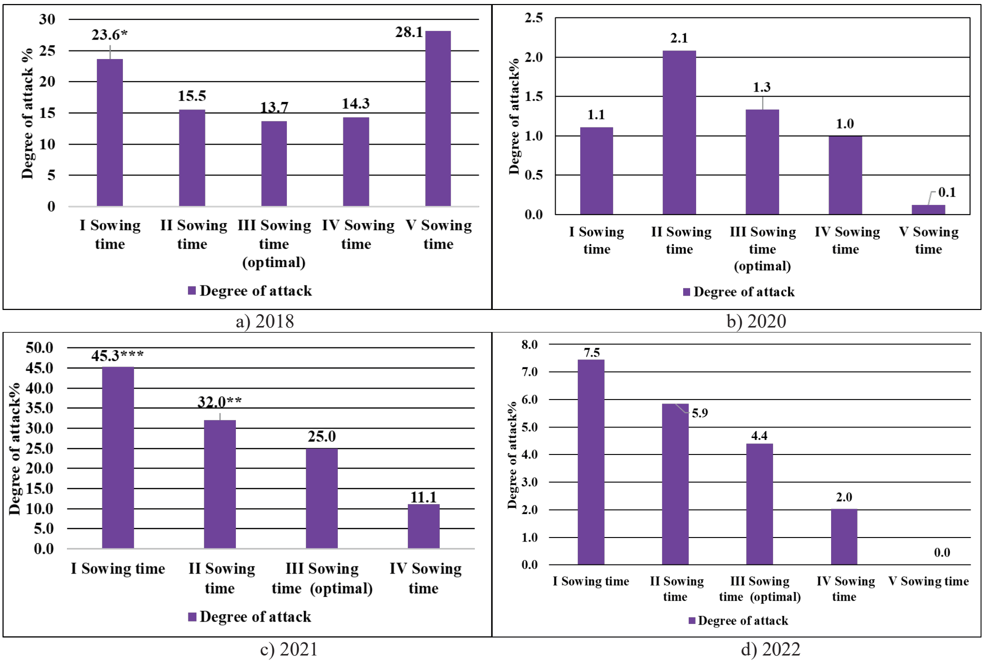
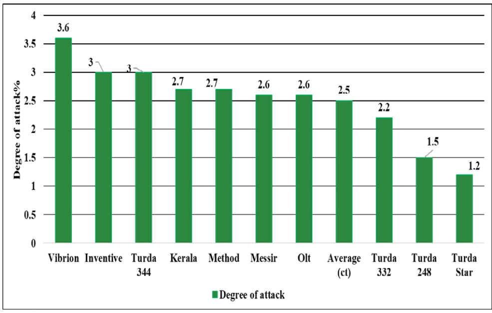


Figure 1. The attack produced by *T. dilaticollis* Gyll. to maize sown in different times (2018-2022)
(Note: LSD where p< 5% = */O; p < 1% **/OO; p < 0.1 % = ***/OOO; 2018: LSD 5%=9.9; LSD 1%=14.0; LSD 0.1%=20.3%; 2020: LSD 5%=1.4; LSD 1%=2.0; LSD 0.1%=2.8; 2021: LSD 5%=13.0; LSD 1%=18.5; LSD 0.1%=26.5; 2022: LSD 5%=2.4; LSD 1%=3.4; LSD 0.1%=5; *T. dilaticollis* Gyll. density: 2018 = 7 specimens/m^2; 2020 = 3 specimens/m^2; 2021 = 5 specimens/m^2; 2022 = 5 specimens/m^2)

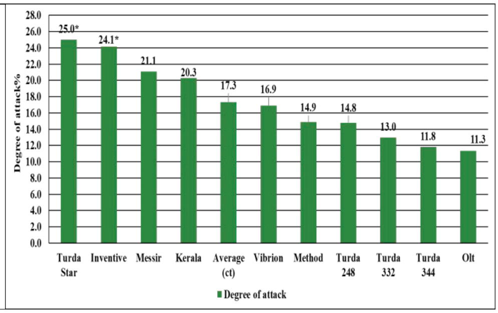
Regarding the weevils attack at some Romanian and foreign maize genotypes, the situation was also influenced by spring weather conditions. In 2020, the attack degree was greatly reduced and ranged from 1.2% (Turda star) to 3.6% (Vibrion). The attack reduction was achieved because the average temperature in May were with -1.5°C lower than the multi-year average (15.4°C) and this conditions were unfavorable for weevils to fed with leaves (Figure 2). The situation was different in 2021, the drought in spring and the high temperatures in the second half of May favored the insects attack. The maize genotypes recorded attacks between 11.3% (Olt) and 25% (Turda star), and the experience average, was of 16.9% (Figure 2).

In 2022, it can be seen that the maximum degree of attack was recorded for the Inventive genotype, of 13.8%, and the lowest, below the experience average (6.0), for Turda 248, of 1.9% (Figure 2).

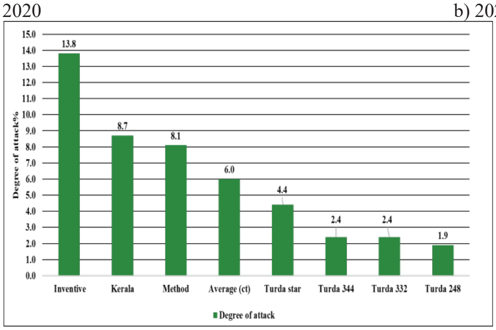
The tested genotypes had the same chemical seed treatment and emerged concomitantly with other maize crops, the adults having other host plants available for feeding, but it is noted that the hybrids Turda 248, Turda 344, Turda Star, Turda 332 and Olt, have registered attack degree values below the experience average.



a) 2020



b) 2021



c) 2022

Figure 2. The attack produced by *T. dilaticollis* Gyll. in some Romanian and foreign maize genotypes (2020-2021)
(Note: LSD where p< 5% = */O; p < 1% **/OO; p < 0.1 % = ***/OOO; 2020: LSD 5%=2.2; LSD 1%=3.2; LSD 0.1%=4.6; 2021: LSD 5%=6.5; LSD 1%=9.2; LSD 0.1%=13.4; 2022: LSD 5%=5.6; LSD 1%=8.0; LSD 0.1%=11.6)

CONCLUSIONS

Climatic conditions in April and May influence the *T. dilaticollis* Gyll. appearance, spread and attack on agricultural crops in Central Moldova.

The most favorable conditions for the insect attack were recorded in April and May 2018, 2021 and 2022 respectively.

The highest degree of attack was recorded on early-emerging maize plants because the hibernating adults concentrated the attack here, compare to the optimal sowing time and the lowest degree of attack values were recorded on late-sown maize.

The genotypes Turda 248, Turda 332, Turda 344, Turda star and olt recorded attack values below the experience average.

It is necessary to continue research regarding *T dilaticollis* Gyll. to identify alternative solutions in order to prevent attacks and control the populations whose densities raise problems year after year in the maize crops in the east of the country.

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WEEDS CONTROL IN INDUSTRIAL HEMP (*Cannabis sativa* L.) BY USING HERBICIDES IN PRE-EMERGENCY AND POST-EMERGENCY

Ioan PUIU[1], Lorena-Diana POPA[2], Carmen Simona GHIȚĂU[1], Costel SAMUIL[1], Constantin LUNGOCI[1], Laurențiu PINTRIJEL[3]

[1]Iasi University of Life Sciences "Ion Ionescu de la Brad" (IULS), M. Sadoveanu Alley 3, Iasi, 700490, Romania
[2]Agricultural Research and Development Station, 377 Principala Street, Secuieni, 617415, Neamt, Romania
[3]Brado Agri S.R.L., Vascauti, 727394, Suceava, Romania

Corresponding author email: constantinlungoci@uaiasi.ro

Abstract

The impressive production potential of industrial hemp (Cannabis sativa L.) is limited by the lack of herbicides authorized for use in this crop in Romania. The aim of this research was to establish the tolerance of industrial hemp to herbicides applied in preemergence and postemergence to control dicotyledonous weeds: Amaranthus retroflexus L., Atriplex patula L., Chenopodium album L., Polygonum convolvulus L., Sinapis arvensis L., Xanthium strumarium L., respectively monocotyledonous: Echinochloa crus-galli L., Setaria spp., Sorghum halepense (L.) Pers. and Poa annua L. A number of 13 herbicides were tested, and the results demonstrate that monocotyledonous weeds can be controlled very well by the active substances cletodim (89.67%) and fluazifop-P-butyl (91.33%), and dicotyledonous weeds are effectively combated by aclonifen (80.0%) applied in pre-emergence and oxyfluorfen (84.0%) applied pre-emergence, but oxyfluorfen causes phytotoxicity (9.0%) of the treated hemp plants. Also, the good effect of clopiralid on Xanthium strumarium and other weeds from the Asteraceae family should be mentioned.

***Key words**: herbicides, industrial hemp, phytotoxicity, weed control.*

INTRODUCTION

Cannabis sativa L., known as industrial hemp, is an herbaceous, annual plant belonging to the Cannabaceae family. Traditionally, hemp plants were primarily cultivated as a fibre crop for the production of textiles and ropes, especially in Eastern Europe and the Western world. Romania was the largest exporter of hemp fibre in Europe before 1989, ranking third globally, and possessed advanced spinning and weaving technology. The cultivated area for industrial hemp in Romania was 49.000 ha before 1990 (Roman et al., 2012). However, in recent years, the actual cultivated hemp acreage has been between 1.000-3.000 ha due to the lack of processing facilities.

Industrial hemp is cultivated for seeds and fibres and has numerous application fields, including food (oil, flour, and de-hulled seed), animal protein feed, animal bedding, cosmetics, industry (textiles, building materials, paper, composites, automotive, pharmacology, etc.) (Farinon et al., 2020). Thanks to its highly developed root system, which surpasses that of other herbaceous plants, hemp is well-suited for phytoremediation of soil contaminated with heavy metals and pesticides (Mihoc et al., 2012). Industrial hemp experts consider it a low-input crop, requiring minimal use of chemicals, fertilizers, water, and pesticides, and it has a positive impact on the environment, contributing to carbon sequestration (Hayo, 2004).

In other crops, cultural practices for weed control, including the use of herbicides, are well-established (Trotus et al., 2020). However, only a limited number of studies have concentrated on weed management strategies with herbicides in hemp production (Flessner et al., 2020). The aim of this research was to identify technological solutions for effectively combating weeds in industrial hemp cultivation using herbicides while minimizing adverse impacts on the environment.

Hemp plants cultivated for seeds are highly susceptible to weed infestation during the initial 30 days of growth, primarily because of the low plant density, which typically ranges from 30 to 40 plants per square meter (Gauca, 2012). There exists a direct correlation between plant density and the effectiveness of weed control (Vera et al., 2002).

The primary goal of this paper is to investigate the degree of herbicide selectivity in hemp fields and the effectiveness of herbicide applications in the control of weed species within hemp cultivation.

Areas under hemp cultivation often exhibit a high degree of weed infestation during the initial growth stages, encompassing both dicotyledonous and monocotyledonous weeds, whether annual or perennial. The prevalence of these weeds varies according to the pedo-climatic conditions in the northeastern region of Romania (Puiu et al., 2022).

In the course of this research, 13 herbicides commonly used in the cultivation of other field crops were tested. These herbicides were applied in accordance with the plant cultivation procedures, both in the pre-emergence and post-emergence stages.

The selection of herbicides was based on a comprehensive review of previous worldwide research pertaining to hemp cultivation and the specific modes of action associated with these herbicides (Laate, 2012; Trotus et al., 2020).

MATERIALS AND METHODS

The experiments were carried out on the non-irrigated fields at the Milen Tech SRL farm, located in Ripiceni village, Botosani county, in order to test a range of 13 herbicides in hemp production (8 pre-emergent herbicides and 5 post-emergent herbicides).

The experimental model: a randomized complete block design with 8 variants and 4 replications for 8 herbicides applied in pre-emergence and a block of 5 variants and 4 replications for 5 herbicides applied in post-emergence (Jitareanu, 1999) (Table 1).

Each experimental plot covered an area of 21 square meters (3.00 x 7.00 meters). Additionally, an untreated check plot was included for comparison.

Table 1. Pre and post-emergent herbicides tested for weed control in hemp production

No	Product	Active ingredient	Product rate, kg, l/ha	Source
Preemergent herbicides				
1	As Super	Metribuzin 700 g/l	1.2	Sharda
2	Challenge 600	Aclonifen 600 g/l	4.0	Bayer
3	Clomate	Clomazone 360 g/l	0.3	Albaugh
4	Dual Gold 960	S-metolachlor 960 g/l	1.5	Syngenta
5	Goal 4 F	Oxifluorfen 480 g/l	0.5	Dow Agro
6	Modown 4 F	Bifenox 480 g/l	1.5	ADAMA
7	Stomp Aqua	Pendimetalin 455 g/l	3.0	BASF
8	Venzar 500	Lenacil 500 g/l	0.8	FMC Agro
Postemergent herbicides				
1	Basagran	Bentazon 480 g/l	2.5	BASF
2	Buctril Universal	Bromoxinil 280 g/l +acid 2,4-D 280 g/l	0.8	Bayer
3	Fusilade Forte	Fluazifop-P-butil 150 g/l	1.2	Syngenta
4	Lontrel 300	Clopyralid 300 g/l	0.5	Dow Agro
5	Select Super	Cletodim 120 g/l	1.5	UPL

The biological material used in the experiment was a monoecious hemp variety with a specific focus on seed production: Secuieni - Jubileu (sourced from the Agricultural and Development Research Station Secuieni in Neamt County, Romania).

The experiment was conducted on cambic chernozem soil with the following characteristics: a pH level of 6.5, a humus content of 2.8%, a nitrogen concentration of 0.22%, a P_2O_5 content of 27 ppm, and K_2O content of 191 ppm.

In the autumn of the year preceding the research, following the harvest of the previous crop (winter wheat), plowing was conducted to a depth of 30 cm, followed by leveling using a disk harrow.

From a climatic perspective, the temperature was in close alignment with the 60-year average for the region. However, notable deviations from the multi-year average were observed in terms of rainfall.

For instance, the month of May experienced abundant rainfall (122.7 mm, compared to the monthly average of 76.9 mm), while the month of August was exceptionally dry (5.8 mm, contrasting with the usual 59.8 mm) (Figure 1).

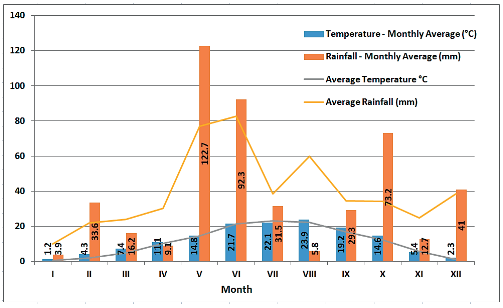

Figure 1. The climatic conditions in Ripiceni in 2020

In the spring, soil preparation for seed germination took place one day before sowing using a harrow. Fertilization was conducted using a complex fertilizer of N:P:K type 15:15:15, with an application rate of 400 kg per hectare, applied during the soil preparation. Additionally, 100 kg per hectare of ammonium nitrate (33.5% nitrogen) was applied during the vegetation phase when the plants had 6 to 8 leaf pairs (BBCH 16-18).

Hemp seeds were sown on April 27, 2020, using a Monosem precision seeder. The planting rate was set at 6 kg per hectare, and the seeds were sown at a depth of 4.0 cm with row spacing of 45.0 cm. The density was 32 plants/square meter.

The pre-emergence herbicides were applied on May 1st, while the post-emergence herbicides were applied 30 days after sowing, when the hemp plants had reached a height of approximately 25-30 cm and they were at the 8-10 leaf pairs stage. The application was carried out using a volume of 250 l/ha.

The evaluation of herbicides' impact on hemp plants included assessments of phytotoxicity and plant vigor. Additionally, the effectiveness of the treatments in controlling the target weeds varied depending on the specific herbicide used.

To assess phytotoxicity, hemp plants were rated for visible injury, including chlorosis and necrosis. The ratings were done on a scale ranging from 0 (no visible injury) to 100% (complete leaves/plants necrosis), following the method described by Fehr et al. (1971).

To evaluate the vigor of the plants, measurements were taken for plant height and leaf mass, with 100% representing values equal to those of the non-treated control.

To determine the effectiveness of herbicides against weeds, a rating system was employed, where grade 0 indicated unaffected weeds and 100% denoted necrotic or dry weeds.

For the herbicides applied pre-emergence and post-emergence, the evaluation was carried out after 2, 4, 6 and 8 weeks after the treatment.

The evaluated effect of herbicides was done for the follow weed species:

- dicotyledonous: *Amaranthus retroflexus* L., *Atriplex patula* L., *Chenopodium album* L., *Polygonum convolvulus* L., *Sinapis arvensis* L. *and Xanthium strumarium* L.;
- monocotyledonous: *Echinochloa crus-galli* L., *Setaria* spp., *Sorghum halepense* (L.) Pers. and *Poa annua* L.

The hemp harvest took place on September 5, 2020. To determine hemp seed yields, samples were collected from a 5.0 square meter area located at the centre of each plot. The seed yield was assessed by manually harvesting. Subsequently, the harvested material was dried at 35°C.

Data were analysed by ANOVA followed by a post hoc Tukey test ($p < 0.05$). Normality and homogeneity of variances were tested before the Tukey test. The program used was IBM SPSS v14.0 (Armonk, NY: IBM Corp.), results were expressed as means.

RESULTS AND DISCUSSIONS

The testing of hemp herbicides in both pre- and post-emergence has revealed significant differences among the herbicides, concerning the tolerance of hemp plants to herbicides, as well as the herbicides efficiency of fighting dicotyledonous and monocotyledonous weeds.

All the herbicides used in pre-emergence show phytotoxicity in hemp, at the first evaluation, (4.67-43.00%), with one exception: Challenge 600 (aclonifen) wich did not cause any visible symptoms in hemp (1.67%).

The highest level of phytotoxicity in hemp, observed 8 weeks after treatment, was associated with As Super (metribuzin) (35.00%) and Clomate (clomazone) (80.67%).

The phytotoxicity of Dual Gold 960 (S-metolachlor) was 25.33% and by Goal 4 F (oxyfluorfen), Modown 4 f (bifenox) and Stomp Aqua (pendimethalin) between 9.00 and 11.00%. In the case of Venzar (lenacil), at the first evaluation the injury level was 14.33%, and after 8 weeks only 6.0% (Figure 2).

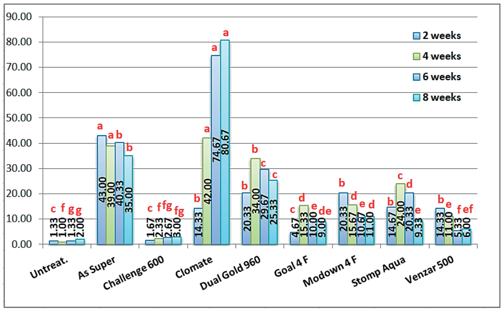

Figure 2. Evaluation of phytotoxicity of hemp treated with pre-emergent herbicides (%)

The height of hemp plants was negatively affected by the majority of the active ingredients used in pre-emergence, especially four weeks after herbicide application. Metribuzin, clomazone, S-metolachlor, oxyfluorfen, bifenox and pendimethalin all led to reductions in plant height. As Super (63.33%) and Clomate (75.33%) were the most harmful (Figure 3).

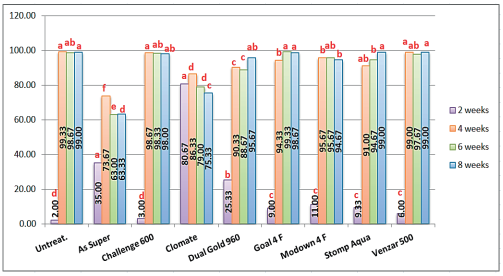

Figure 3. Evaluation of vigour of hemp treated with pre-emergent herbicides (%)

Out of the 8 active ingredients tested pre-emergence, it is notable that aclonifen (80.0%) and oxyfluorfen (84.0%) exhibited effective control over the studied dicotyledonous weeds. Oxyfluorfen, despite its efficacy, had a phytotoxic effect on hemp plants.

In the case of metribuzin, it effectively controlled dicotyledonous weeds. However, it is crucial to note that metribuzin cannot be used for hemp cultivation because it has a severe adverse impact on hemp plants. On the other hand, the active substances clomazone, s-metolachlor, bifenox, pendimethalin and lenacil did not offer sufficient protection for hemp.

The herbicide s-metolachlor effectively controls monocotyledonous weeds in the early stages of hemp growth and is relatively well tolerated by hemp plants, with plant vigor ranging between 88.67% and 95.67% (Figure 4).

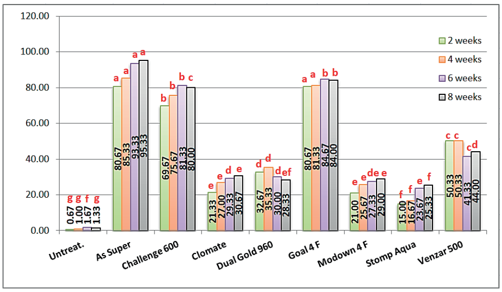

Figure 4. Evaluation of efficiency of pre-emergent herbicides against weeds (%)

Among the post-emergence herbicides used in hemp, including Basagran, Buctril Universal, Fusilade Forte, Lontrel 300 and Select Super, they initially exhibited phytotoxic effects on hemp during the first 2 weeks after treatment, with phytotoxicity ranging from 1.0% to 34.0%. However, after 8 weeks of treatment, bentazon caused severe damage to hemp plants (65.0%), while bromoxynil had a lesser impact (14.67%). Fluazifop-p-butyl, clopyralid, and clethodim no longer affected hemp plants (Figure 5).

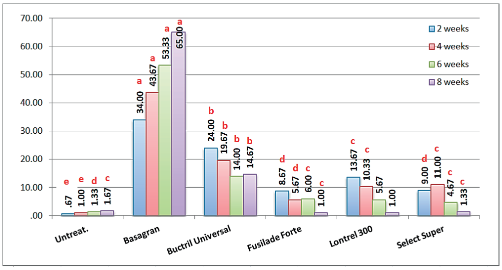

Figure 5. Evaluation of phytotoxicity of hemp treated with post-emergent herbicides (%)

Regarding the hemp vigour, at the first evaluation, the plants are affected in all variants (75.33-95.67%) (Figure 6).

After 8 weeks from herbicides application, only the hemp treated with the active ingredients bentazon (69.00%) and bromoxynil (90.33%) exhibites reductions in height.

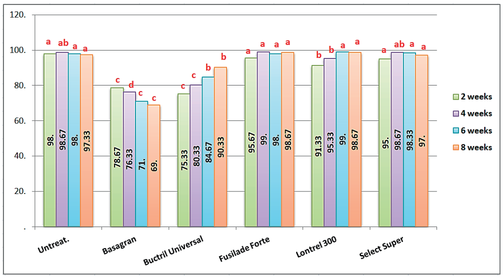

Figure 6. Evaluation of vigour of hemp treated with post-emergent herbicides (%)

This sugests that the hemp plants can recover from the impact of some harmful herbicides during the phases following application.

For the control of monocotyledonous weeds, the ingredients fluazifop-p-butyl (91.33%) and clethodim (89.67%) recorded a high efficiency and clopyralid controlled 61.0% of dicotyledonous weeds (especially weeds from the *Asteraceae* family, eg *Xantium strumarium* L., the most spread and harmful weed in hemp fields).

Bentazon and bromoxynil effectively combated dicotyledonous weeds. However, it's important to note that these herbicides are phytotoxic to hemp and are not suitable for use in hemp cultivation (Figure 7).

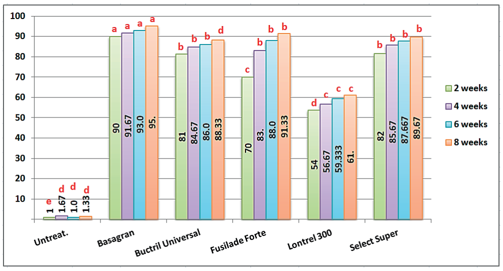

Figure 7. Evaluation of efficiency of post-emergent herbicides against weeds (%)

The control of dicotyledonous weeds in post-emergence is very difficult in hemp, because the plants are very sensitive to the application of specific herbicides.

The monocotyledonous weeds can be effectively combated by specific herbicides, because these are selectiv for hemp and have low phytotoxicity.

The production of hemp seeds varied depending on the effectiveness of the herbicide in controlling weeds and the selectivity of the products against hemp plants.

In the case of pre-emergence herbicides, the highest seed production was observed in the variant treated with Challenge (989.0 kg/ha), followed by Goal 4 F (949.7 kg/ha) and Dual Gold 960 (882.7 kg/ha). The untreated check yielded 766.3 kg/ha (Table 2).

Table 2. The influence of pre-emergent herbicides on hemp grain yield

Plots	Yield (kg/ha)	Difference (%)	Difference (kg)	Meaning
Challenge 600	989.0	129.06	222.7	***
Goal 4 F	949.7	949.7	183.4	***
Dual Gold 960	882.7	882.7	116.4	***
Untreat.	766.3	766.3	0.0	unt.
Stomp Aqua	650.7	650.7	- 115.6	ooo
Modown 4 F	630.7	630.7	- 135.6	ooo
As Super	570.3	570.3	- 196.0	ooo
Venzar 500	542.0	542.0	- 224.3	ooo
Clomate	443.3	443.3	- 323.0	ooo
LSD $_{5\%}$	14.2	kg/ha		
LSD $_{1\%}$	19.6			
LSD $_{0.1\%}$	27.0			

Among the post-emergence herbicides, Lontrel 300 resulted in the highest seed production (936.7 kg/ha), followed by Select Super (911.7 kg/ha) and Fusilade Forte (893.0 kg/ha). The untreated check yielded 741.7 kg/ha (Table 3).

Table 3. The influence of post-emergent herbicides on hemp grain yield

Plots	Yield (kg/ha)	Difference (%)	Difference (kg)	Meaning
Lontrel 300	936.7	126.29	195.0	***
Select Super	911.7	122.92	170.0	***
Fusilade Forte	893.0	120.40	151.3	***
Untreat.	741.7	100.0	0.0	unt.
Buctril Universal	682.0	91.35	-59.7	ooo
Basagran	521.7	70.34	-220.0	ooo
LSD $_{5\%}$	13.8	kg/ha		
LSD $_{1\%}$	19.6			
LSD $_{0.1\%}$	28.3			

In both application variants of the mentioned herbicides, seed productions are notably higher compared to the untreated control.

CONCLUSIONS

The herbicides tested on hemp, applied pre- and post-emergence in the field, exhibited varying levels of selectivity towards hemp and efficiency in controlling mono- and dicotyledonous weeds.

Throughout the research, selective herbicides were identified that provide effective weed control, while protecting hemp in the field.

Among the herbicides applied in pre-emergence, Challenge 600 herbicide was well-tolerated by hemp and proved to be highly effective. Goal 4 F also effectively controlled dicotyledonous weeds and was selective enough to allow for robust hemp production. Dual Gold 960 can be considered for protecting hemp crops as well.

On the other hand, As Super and Clomate herbicides exhibited significant phytotoxicity towards hemp. Additionally, Modown 4 F, Stomp Aqua, and Venzar 500 were not very efficient in weed control, and it is advisable to avoid their use in hemp cultivation.

In post-emergence applications, Lontrel 300 effectively controlled dicotyledonous weeds, while Select Super and Fusilade Forte were successful in combating monocotyledonous weeds. However, herbicides such as Buctril Universal and Basagran were phytotoxic to hemp and should not be used in hemp production.

The highest grain production was achieved in tests with pre-emergence herbicides, particularly Challenge 600 (989.0 kg/ha) and Goal 4 F (949.7 kg/ha), surpassing the yield of the untreated check, which was 766.3 kg/ha.

In the case of post-emergence herbicide applications, the best production results were seen in the variant with Lontrel 300 (936.7 kg/ha), followed by Select Super (911.7 kg/ha). Again, the untreated check yielded 741.7 kg/ha.

Based on the findings, a strategy for protecting hemp crops against weeds can be developed. This strategy includes the use of a pre-emergence herbicide like Challenge 600 or Goal 4 F, in addition to two post-emergence herbicides applied separately. These post-emergence herbicides can be Select Super or Fusilade Forte to combat monocotyledonous weeds and Lontrel 300 to control dicotyledonous weeds.

Furthermore, it's essential to implement all the necessary technological steps to protect the hemp crop during the initial phases of growth. This may involve crop rotation, mulching, selecting the right sowing date, adjusting the seed rate per hectare, and employing mechanical weeding as needed.

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TESTING THE INSECTICIDAL ACTIVITY OF PLANT EXTRACTS FOR THE CONTROL OF ECONOMICALLY IMPORTANT ENEMIES OF RAPE FROM THE ORDER *Coleoptera*

Stefan RASHEV[1], Nedyalka PALAGACHEVA[2], Stoyan GEORGIEV[1]

[1]Field Crops Institute Chirpan, Agricultural Academy Bulgaria,
2 Georgi Dimitrov Blvd, Chirpan Bulgaria
[2]Agricultural University of Plovdiv, 12 Mendeleev Blvd, Plovdiv, Bulgaria

Corresponding author email: stogeorgiev@abv.bg

Abstract

Rapeseed is attacked by a number of enemies, of which the rapeseed weevil (Meligethes aeneus F.) and the pod borer (Ceutorhynchus assimilis Payk.) are of important economic importance. They damage the generative organs, which is why mining is directly dependent on the organization and effectiveness of the fight against them. In recent years, the mass use of chemical means to combat them leads to the development of resistance, the destruction of useful species and pollinators of cultivated plants. In order to limit their application, alternative means of control are sought to avoid the negative consequences of the use of chemical preparations. In this regard, the efficacy of plant extracts from: walnut (Juglans regia L.), wild walnut (Ailantthus altissima Swing.) and tobacco (Nicotiana tabacum L.) against the adult forms of the rape blossom borer and the pod beetle was tested under laboratory conditions. The attempt was made in four variants and three repetitions. The obtained results show that the compounds contained in the plant extracts exhibit efficacy against the adults of the rape flower borer and the pod borer. The obtained results are a prerequisite for the application of the plant extracts in the integrated systems to combat the enemies of rape.

Key words: *Meligethes aeneus F., Ceutorhynchus assimilis Payk., plant extracts, insecticide activity.*

INTRODUCTION

The canola borer is the most economically important enemy of canola, which can reduce yields by up to 80%. The species appears in rape fields in early spring (BBCH 51-57) and causes the greatest damage in the phenophase of budding-flowering (BBCH 57-60) (Cool et al., 1998; Hovorka et al., 2021). According to Tarang et al. (2004) the main damage is caused by adults. At a density of 1.4-2.5 beetles per plant, seed yield decreases by 3.6 t/ha.

Despite the fact that hidden octopuses appear in calamities relatively less often, they represent a serious danger to the trap. Their losses become obvious when they cannot take any measures to prevent losses (Arabadzhiev, 1959). Overwintering adults migrate to canola in the flowering phenophase (BBCH 61-65), females lay eggs singly in pods (Lerin, 1991) and hatched larvae develop within them (Williams, 2010).

According to Free and Williams (1978), the pod octopus prefers to lay its eggs in pods already damaged by the tropical flower-eater. Fergusan et al. (2003) found that the canola flower eater and the pod octopus meet complexly in the crop and are key enemies of canola (Hovorka et al., 2021).

Essential oils and phytochemicals isolated from plants of the Apiaceae family exhibit good insecticidal activity and inhibitory action against eggs, larvae and adults and can be used in practice to control a number of crop pests (Ebadollahi, 2013).

In his research, Pavela (2011) tested the insecticidal effect of essential oils from 9 plants: *Carum carvi* L., *Cinnamomum osmophloeum* Kaneh., *Citrus aurantium* L., *Foeniculum vulgare* Mill., *Lavandula angustifolia* L., *Mentha arvensis* L., *Nepeta cataria* L., *Ocimum basilicum* L., *Thymus vulgaris* L. against the adult forms of rape blossom borer. All essential oils tested showed high efficacy and caused death of adult insects. The highest efficacy, 65.6%, was found in the extracts of caraway (*Carum sarvi* L.) and thyme (*Thymus vulgaris* L.) 63.8%.

A number of authors Pavela (2005, 2006, 2009), Pavela et al. (2009a, 2009b), Zabka et

al. (2009), Nerio et al. (2010) in their research found that essential oils extracted from plant species exhibit insecticidal, bactericidal and fungicidal action.
According to Isman (2000), Nerio et al. (2010) essential oils in most cases are characterized by repellent action against pests.
Hummelbrunnerand Isman (2001) and Pavela (2008) found that essential oils of plant origin could not only cause mortality, but also affect the fecundity and lifespan of predators.

MATERIALS AND METHODS

The studies were carried out under laboratory conditions at the Agricultural University - Plovdiv. The insecticidal action of the following plant extracts: walnut (*Juglans regia* L.), wild walnut (*Ailantthus altissima* Swing.) and tobacco (*Nicotiana tabacum* L.) was tested against the adults of the rape flower borer and the pod borer.
The leaves, stems and flowers of the above plants were soaked for 24 hours in a ratio of 100 mg plant mass: 100 ml water, after which the working solutions were prepared.
The experiment was carried out in three repetitions and four variants: Variant I - wild walnut extract, Variant II - tobacco extract, Variant III - a combination of wild walnut and tobacco, Variant IV - control. In each variant, 10 adult canola borer and pod borer insects, food (canola flowers) were placed in plastic containers and the insects were sprayed with the aqueous emulsions of the plant extracts.
Readings were performed on the 3rd, 5th and 7th day.
The experimental data were compared with the mean values of independent samples at a significance level of $p \leq 0.05$.

RESULTS AND DISCUSSIONS

The results of the conducted research show a clear distinction of the control (Var. 1) from the other variants, which is evident from the variance analysis performed and the applied comparison of the average values.
A statistically proven stronger effect of the extracts on the pod weevil than on the rapeseed weevil was observed, at a significance level of p = 0.002084 (Figure 1).

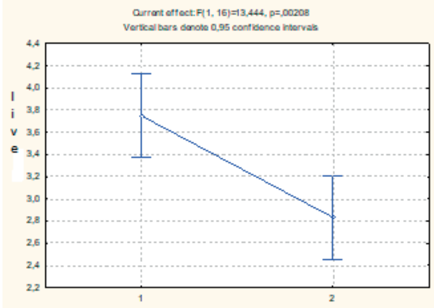

Figure 1. Comparison of impact on enemies - rape flower eater and pod borer

There is a statistical difference in the degree of impact of the individual extracts at a significance level of p = 0.00005 (Figure 2).

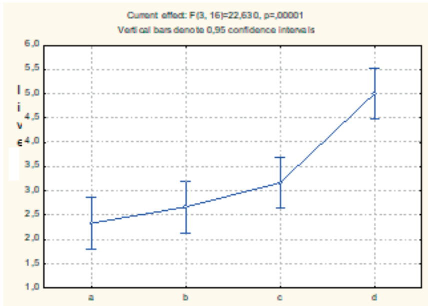

Figure 2. Effect of the extracts: a - tobacco; b - wild walnut; c - tobacco + wild walnut; d - control

With the enemy - extract combination, there is also a proven statistical difference at a significance level of p = 0.002332 (Figure 3). This gives reason to claim that, at this stage of the ongoing research, each extract has a different effect on the mortality of different enemies.

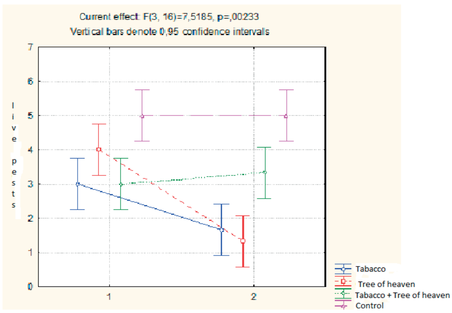

Figure 3. Influence of the combination of the factors on the vitality of the enemies.

There is only a proven statistical difference for the effects of the extracts, at a significance level of p = 0.000001. There is no difference between the individual extracts in their effect on the enemies. The difference is between each one of them and the control (no treatment - Figure 4).

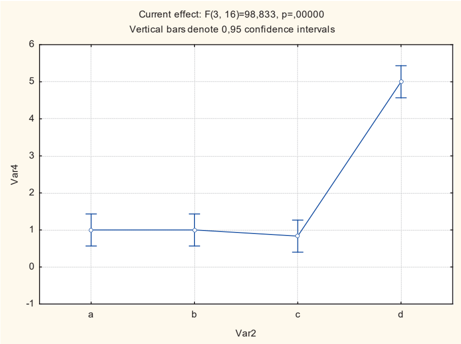

Figure 4. Comparing the effect of extracts and no treatment

On the 5th day the picture is the same as on the 3rd day.

There is a proven statistical difference at a significance level of p = 0.003276 in the effect of the extracts compared to the control (Figure 5) at the 24th hour. At this stage, tobacco extracts and the combination of tobacco and wild walnut have a stronger effect, but without statistical proof.

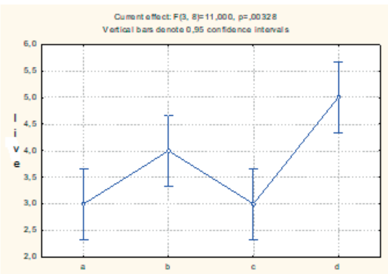

Figure 5. Effect of the extracts: a - tobacco; b - wild walnut; c - tobacco + wild walnut; d - control on the rape flower borer at the 24th hour

There is a proven statistical difference at a significance level of p = 0.000081 in the effect of the extracts compared to the control (Figure 6) on the 3rd day. At this stage, all extracts have the same degree of effect.

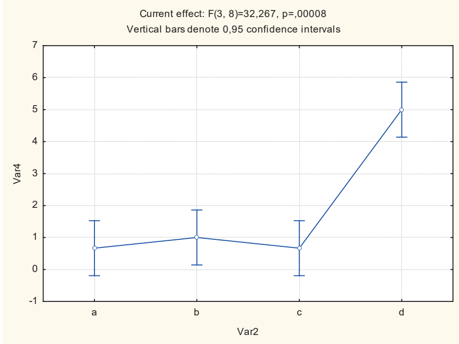

Figure 6. Effect of the extracts: a - tobacco; b - wild walnut; c - tobacco + wild walnut; d - control on the rape flower borer on the 3rd day

CONCLUSIONS

As a result of the conducted studies, the following more important conclusions can be drawn:

1. The compounds contained in the plant extracts of walnut (*Juglans regia* L.), wild walnut (*Ailantthus altissima* Swing.) and tobacco (*Nicotiana tabacum* L.) showed higher efficacy against adults of the pod octopus (*Ceutorhynchus assimilis* Payk.). Among them, the highest tobacco extracts and the combination of wild walnut and tobacco show efficacy.
2. The obtained results are a prerequisite for the application of the plant extracts in the integrated systems for combating the enemies of rape, protecting the beneficial entomofauna and the pollinators of the cultivated plants.

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PHOTOSYNTHETIC PROCESSES AND USES DIFFERENT FORMS OF FATTY ACIDS IN CORN PLANTS DURING THE PERIOD OF INTENSIVE GROWTH AND UNDER THE INFLUENCE OF FERTILIZER AND GROWTH REGULATOR

Yosyp RIVIS[1], Vasyl FEDAK[1],Oksana MAMCHUR[2], Volodymyr BALKOVSKYI[3], Ivan SHUVAR[3], Olena BEZALTYCHNA[4], Bogdan GUTYJ[5], Olha STADNYTSKA[1], Hanna KORPITA[3], Antin SHUVAR[1], Khrystyna LESKIV[5]

[1]Institute of Agriculture of the Carpathian Region of NAAS of Ukraine, 5 Grushevskogo Street, Obroshino, 81115, Ukraine
[2]Ivan Franko National University of Lviv, 1 Universytetska Street, Lviv, 79000, Ukraine
[3]Lviv National Agrarian University, 1 V. Velykiho Street, Dublyany, 80381, Ukraine
[4]Odessa State Agrarian University, 13 Panteleymonyvska Street, Odessa, 65012, Ukraine
[5]Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies Lviv, 50 Pekarska Street, Lviv, 79010, Ukraine

Corresponding author email: bvh@ukr.net

Abstract

The aim of the research was to identify quantitative changes in the processes of photosynthesis and fatty acid composition of the maize plants vegetative mass during ontogenesis and under the influence of mineral fertilizers and growth regulators. It is established that the functional activity of the photosynthetic apparatus in the leaves of maize plants increases with their age and depends on soil fertilizer and the influence of growth regulators. It was found that the increase in the functional activity of the photosynthetic apparatus of maize plants in the phase of intensive growth is accompanied by an increase in the concentration of esterified fatty acids and an increase in the ratio of more valuable linolenic polyunsaturated fatty acid to less valuable linoleic polyunsaturated fatty acid in stems and leaves. It is established that the formation of generative organs of maize plants leads to a gradual decrease in the concentration of esterified fatty acids and a decrease in the ratio of linolenic polyunsaturated fatty acid to linoleic polyunsaturated fatty acid in the stem and leaves.

Key words: *corn, phases of vegetation, fertilizer, regulator of growth, photosynthetic processes.*

INTRODUCTION

The coordinated functioning of biological membranes ensures plant cell homeostasis. Along with proteins, the main components of natural membranes are lipids and fatty acids. The action of exogenous or endogenous factors induces a change in the conformation of these molecules. Thus, the course and direction of metabolic functions in the cell change. The level of fertilization and biologically active substances play an essential role in the regulation of metabolic processes in the cell (Kramarov, 2003; Kovalchuk et al., 2019; Sobolev et al., 2020).

However, there needs to be more data on the influence of the level of fertilizer and biologically active substances of different classes on the metabolism of lipids and fatty acids, particularly in plants, in the scientific literature. In individuals, the effect of fertilizer and growth stimulants on the metabolism of phospholipids, primarily phosphatidylcholine, in the plasma membrane of corn seedlings has been shown (Storozhenko, 2004; Dalal & Tripathy, 2012). There are also incomplete reports on the metabolism of fatty acids in the ontogeny of corn (Bates et al., 2013; Wang et al., 2020). In these studies, it was established that the fatty acid composition of lipids in the leaves changes due to the proportion of linoleic acid during the corn-growing season.

Based on the above, the goal of our work was to reveal quantitative changes in the processes of photosynthesis and the fatty acid composition of the vegetative mass of corn plants during the period of intensive growth and under the influence of mineral fertilizers and growth regulators.

MATERIALS AND METHODS

In the research, a simple mid-ripening corn hybrid Zbruch (FAO-310) was used, which was entered into the State Register of Plant Varieties of Ukraine in 2008. The hybrid is recommended for cultivation and is zoned in the Forest Steppe and Steppe. This maize hybrid of intensive type with high yield potential responds well to improved growing conditions. The main morphometric characteristics of hybrid plants: the average height of the plant is 210-220 cm, the length of the cob is 20-22 cm, the height of the cob attachment is 70-80 cm, the cob is cylindrical with a red stem, contains 14-16 rows of grains and 38-40 grains in a row. The kernel is yellow and tooth-shaped. The average weight of 1,000 kernels is 280-290 g. Corn plants of the Zbruch hybrid are characterized by resistance (out of 9 points): before lodging - 8; to the cold - 8; before drought - 8; before being affected by the major diseases - 8; before damage by pests - 8. The grain yield is 11.0-13.0 t/ha.

The plant growth regulator Zeastimulin, developed by the Research Institute of Bioorganic Chemistry and Petrochemistry of the National Academy of Sciences of Ukraine, was also used in the research, and its effectiveness meets world standards. According to the sanitary and hygienic classification, this drug belongs to low-toxic substances of the III-IV hazard class (GOST 12.1.007-76), has a positive effect on the microflora, and is quickly transformed by soil microorganisms and plant cells (Ponomarenko, 2003).

Zeastimulin (TU U 88.264.036-97) is a balanced composite plant growth regulator containing a complex of ivin with formic acid in combination with emistim C. It is a colorless liquid with a weak alcohol smell, unlimitedly soluble in water and polar solvents. The Ukrgosphimkomisi registers it as a corn growth regulator. The drug helps to increase the yield of corn grain by 7-10 t/ha, green mass - by 50-70 t/ha and increases the number of fats and proteins in the grain.

Field research was carried out in the barley-clover crop rotation (plowing of the II slopes) - winter wheat-corn of the laboratory of grain and fodder crop seed production of the Institute of Agriculture of the Carpathian region of the National Academy of Sciences. For liming, we used limestone flour (GOST 14050-93) with a content of $CaCO_3$+$MgCO_3$ - 93.5% of local production (JSC Pustomytivske Zavodopravlinia Vapnovyh Zavod).

The experiments were conducted on gray forest surface-glazed soils. The following agrochemical parameters characterize the arable layer (0-20 cm) of the soil: the pH of the salt extract is 4.8-5.2, the content of humus (according to Tyurin) is 2.26-2.53%, the content of easily hydrolyzed nitrogen is 9.24- 12.6 mg per 100 g of soil, mobile Phosphorus (according to Kirsanov) - 7.11-9.8 mg, exchangeable Potassium (by flame photometry) - 10.2-12.2 mg/100 g of soil.

Weather conditions during the experiment generally contributed to the formation of a high yield of green mass of corn and grain.

The technology of growing corn in the experiment is generally accepted for the soil and climate zone conditions. The predecessor is winter wheat with the harvesting of post-harvest residues and sowing of siderat (oil radish) with subsequent plowing for frost (25-27 cm).

The scheme of the experiment has the following form: 1 - control (without fertilizers), 2 - experiment (fertilizer $N_{60}P_{45}K_{45}$), 3 - experiment (growth regulator Zeastimulin). The sowing area is 39 m^2, and the accounting area is 25 m^2.

Corn seeds were sown in wide rows with a row width of 60 cm to a depth of 5-6 cm. Sowing was carried out from the end of April - the beginning of May. The optimal temperature of warming the soil was at a depth of 10 cm to 8-10ºC, which contributed to the appearance of friendly seedlings at a rate of 63,000 units/ha (50-55 thousand units/ha + 15% insurance premium - 63 thousand units/ha).

For the first spring cultivation, mineral fertilizers of the following composition were applied: ammonium nitrate (34% N), granulated superphosphate (19.5% P), and potassium salt (40% K) in a dose $N_{60}P_{45}K_{45}$. In the II experimental plot, corn plants in the 5-6 leaf phase were treated with an aqueous solution of the growth stimulator Zeastimulin (20 ml/ha) according to the manufacturer's recommendations. In addition, the plants were treated using a knapsack sprayer. The consumption of the mixture was 200 l/ha, and the control plants were treated with distilled water.

Phenological observations were carried out according to the instructions "Conducting and state testing of plant varieties of grain, cereal and leguminous crops" with the following phenophase of vegetation: seedling, shedding of panicles, the beginning and full flowering of panicles and cobs.

Harvesting and accounting of the harvest of green mass of corn were carried out during the phenophase of 8-9 leaves, panicle shedding, and cob flowering.

The content of chlorophylls a and b were determined in plant leaf samples taken during the phenophase of 8-9 leaves, panicle shedding, cob flowering, and non-esterified and esterified fatty acids in stem and leaf samples taken during ontogenesis. The content of chlorophyll a and c was determined spectrophotometrically (Deeva, 2008). For this, pigments were extracted from medium leaf samples with acetone (according to Holm-Wettstein), and the resulting solutions were spectrophotometer. Determination of the content of non-esterified (so-called free) and esterified (found in the studied material in the composition of phospholipids, esterified phytosterol, mono-, di- and triacylglycerols) fatty acids was carried out by the method of gas-liquid chromatography (Rivis, 2017). For this, total lipids from the average samples of stems and leaves were extracted with a chloroform-methanol mixture (2: 1 by volume). First, the obtained lipids were dissolved in hexane. Next, a solution of sodium metal in methyl alcohol was added to the hexane solution of lipids in a test tube, and after that, the test tube was vigorously shaken. The methyl esters of fatty acids obtained in this way were injected into the evaporator of the gas-liquid chromatographic apparatus using a microsyringe. Peaks of esterified fatty acids were obtained on chromatograms.

Next, a few drops of glacial acetic acid and a few milliliters of hexane were added to the sediment in the test tube. After that, the test tube was intensively shaken. After its contents stratification, the upper hexane layer was removed with a pipette with a retracted nozzle and transferred to a special test tube with a grinding wheel. Hexane from this test tube was evaporated in a vacuum cabinet at a temperature of 40-45°C. A few drops of methyl alcohol and, as a catalyst, a few drops of acetyl chloride were added to the fatty acids. After that, the test tube was closed with a polished cork and placed for one and a half hours in a converted thermostat, in which the upper, larger part of the test tube was cooled by running water from the tap, and the lower, smaller part was in a water bath at the boiling temperature of methanol (65°C). After completion of methylation, the remaining methanol in the test tube was evaporated in a vacuum cabinet at a temperature of 40-45°C. The methyl esters of fatty acids obtained in this way were dissolved in a few drops of hexane and introduced into the evaporator of the gas-liquid chromatographic apparatus using a microsyringe. Peaks of non-esterified fatty acids were obtained on the chromatograms. To obtain quantitative data on esterified and non-esterified fatty acids, internal normalization and standard internal methods were used.

Field experiments were carried out over three years in four repetitions. Biochemical experiments were carried out in three biological and three analytical replicates. The obtained digital material was processed by the method of variational statistics using the Student's criterion. Average arithmetic values (M) and their errors (± m) were determined. Changes were considered probable at $p<0.05$. The computer program Origin 6.0, and Excel (Microsoft, USA) was used for calculations.

RESULTS AND DISCUSSIONS

The pigment system of higher plants consists of many optically active substances, but only pigments localized in chloroplasts are directly involved in photosynthesis (Andrianova & Tarchevsky, 2000).

The high functional activity of chloroplasts is the essential feature of the formation of the photosynthetic apparatus, which determines the level of the plant's vital activity in general and contributes to its better response to optimal conditions. As a result, to obtain high yields since it is known that between the number of pigments and the productivity of plants, their viability, and stability, there is a direct dependence on adverse environmental factors (Guidi et al., 2019).Furthermore, the level of mineral nutrition and the effect of growth regulators significantly influence the formation, structure, and activity of the photosynthetic apparatus of plants (Kramarov, 2003; Fromme et al., 2019; Ali, 2021).

It was established that different types of plants, depending on environmental conditions, have different chlorophyll content (Chotewutmontri & Barkan, 2016). Plants form a system that ensures the creation of the most optimal synthesizing apparatus in specific conditions, the main parameters of which can become criteria for optimality or inadequacy of growth conditions (Dalal & Tripathy, 2012). Studies of the dynamics of the content of chlorophyll *a*, a molecule directly involved in the accumulation of light energy, and chlorophyll *b*. The molecule *a,* indirectly related to photosynthesis, allows us to estimate the volume of energy conversion by chloroplast membranes during photosynthesis (Cook et al., 2021). The transformation of chlorophyll a into chlorophyll b is reversible, which allows to quickly change both the total content of chlorophyll in the plant and the ratio of chlorophyll a to chlorophyll b (Ruan et al., 2017).

In the field, we investigated the content of chlorophylls *a* and *b* in the leaves of Zbruch hybrid corn during the period of intensive growth and under the influence of the level of fertilizer and the growth regulator Zeastimulin. It was established that during the period of intensive growth of corn plants, the content of chlorophyll in leaves naturally increases (Table 1), which is consistent with existing facts that indicate the accumulation of the maximum content of photosynthetic pigments in plant leaves before the beginning of their flowering (Shumskaya & Wurtzela, 2013; Li et al., 2020).

Table 1. Chlorophyll content in the leaves of Zbruch hybrid corn plants during the period of intensive growth, $g \cdot 10^{-3}$/kg of natural weight (M ± m, n = 3)

The investigated indicators	Control	Experiment	
		$N_{60}P_{45}K_{45}$	Zeastimulin
Phase 8-9 leaves			
Chlorophyll *a*	209.2±3.24	224.9±16.31	246.2±20.37*
Chlorophyll *b*	60.2±0.59	61.5±1.23	73.0±1.53*
Sum *a* i *b*	269.5±2.72	286.3±8.49	319.2±10.33*
a/b	3.54	3.67	3.38
The phase of throwing out the panicle			
Chlorophyll *a*	211.4±2.02	233.0±3.36*	259.5±3.41*
Chlorophyll *b*	59.9±0.85	60.7±0.52	70.7±0.44*
Sum *a* i *b*	271.0±1.25	291.5±1.07*	330.2±3.57*
a/b	3.51	3.86	3.67
Flowering phase of cobs			
Chlorophyll *a*	214.9±2.12	240.6±1.23*	263.1±5.41*
Chlorophyll *b*	59.9±0.47	61.1±0.44	68.8±0.20*
Sum *a* i *b*	274.8±2.32	301.7±1.74*	331.3±5.54*
a/b	3.59	3.93	3.82

Note: here and hereafter* p<0.02-0.05, ** p<0.01, *** p<0.001.

The level of soil fertilization, along with other exogenous factors, has a significant impact on the formation, structure, and activity of the photosynthetic apparatus of plants, which is quantitatively expressed in the increase in the content of chlorophyll. The content of chlorophyll in the leaves of corn due to the action of fertilizers compared to the control increases by 7-12% during the growing season, ensuring a maximum increase in the number of pigments in the flowering phase by 10% (Table 1).

It is worth noting that under these conditions, chlorophyll turned out to be less sensitive to the conditions of soil fertilization. Therefore, its content practically did not change during the researched vegetation phase and remained at control values. Along with this, due to the effects of fertilizers, an increase in the ratio of the content of chlorophyll a to chlorophyll c in the investigated vegetation phase by 7-10% and by 3-9% compared to the control was found, which indicates a better adaptation of corn plants to external influences in these conditions.

Changes in the content and ratio of photosynthetic pigments detected by us under the action of fertilizers are consistent with data from the literature (Zhu et al., 2014; Fromme et al., 2019). In addition, the components of the pigment fund of plants react differently to the lack of mineral nutrition. Thus, the chlorophyll content under lack of mineral nutrition, especially nitrogen, sharply decreases, which is visually manifested in plant chlorosis (Dalal & Tripathy, 2012).

Biologically active substances of various nature cause changes in the pigment fund of leaves, remarkably increasing chlorophyll content (Pshibytko et al., 2003; Wang et al., 2018). Our research results prove that treating corn plants with the growth regulator Zeastimulin caused additional activation of biosynthesis and accumulation of chlorophyll *a* and chlorophyll *c* in plant leaves.

The use of exogenous phytohormones, which shift the phytohormonal balance of the plant in the direction of stimulator phytohormones, helps to activate the functioning of the photosynthetic apparatus, increase the stability of the pigment complex of plants and, therefore, the productivity and yield of plants, as evidenced by the literature (Wang et al., 2018; Guidi et al., 2019).

It was established that the content of chlorophyll *a* in the leaves of corn plants under the influence of the growth regulator Zeastimulin increases during intensive growth by 17-22% compared to the control. In contrast to the effect of soil fertilization, under the influence of Zeastimulin, an increase in the chlorophyll *b* content of these plants was also found - by 16-20% compared to the control values in the corresponding phases of vegetation, which can be explained by the onto genetic adaptation of plants in these conditions (Guidi et al., 2019). The better ontogenetic adaptation of corn plants is also indicated by a slight (6-10%) increase in the ratio of chlorophyll *a* to chlorophyll *b* content in the panicle shedding and plant flowering phases compared to the corresponding control values.

Thus, optimal conditions for the biosynthesis and accumulation of chlorophyll in the leaves of Zbruch hybrid corn plants were created under the influence of Zeastimulin. Somewhat lesser extent - under the impact of the applied mineral fertilizer, which contributed to the effective functioning of the pigment system during intensive growth, high intensity of photosynthesis, and, respectively, accumulation of raw and dry mass.

Positive changes in the content of non-esterified and esterified forms of fatty acids in the stem and leaves of corn leaves during the period of intensive growth accompany the detected changes in the concentration of chlorophyll *a* and *b*.

Non-esterified fatty acids in the stem and leaves of corn are primarily included in the composition of phospholipids, which in turn are used to form cell membranes (Qin et al., 2013). At the same time, biologically active substances are synthesized in plant tissues from non-esterified fatty acids, primarily oxylipins (Wang et al., 2020). Secondly, non-esterified fatty acids in the composition of triacylglycerols are deposited in the stem and leaves of corn (Wang et al., 2005).

Among other forms of fatty acids, non-esterified ones have significant metabolic and energetic activity, high lability, and reactivity. Based on the above, the quantitative and qualitative composition of non-esterified fatty acids in plant cells and tissues is susceptible to their energy and metabolic needs and readily responds to changes in the homeostasis of a cell, organ, or organism.

Given the importance of the functions performed by non-esterified fatty acids in the plant body, in the conditions of field research, we studied the peculiarities of changes in their content in the green mass of corn plants. The phases of the formation of above-ground vegetative organs, shedding of panicles, and flowering of panicles and cobs constitute the period of intensive growth of corn (Guidi et al., 2019). We found that during the intensive growth phase of corn plants, the content of non-esterified forms of saturated, monounsaturated, and polyunsaturated fatty acids in the control areas in its stem and leaves gradually and slightly decreases (Tables 2, 3, and 4). It can be seen due to their greater inclusion in the composition of phospholipids, esterified cholesterol, and triacylglycerols. The content of non-esterified saturated and polyunsaturated substances sharply decreases only in the flowering phase of the cobs. Perhaps this is related to the formation of grain cell membranes.

It should be noted that during the flowering phase of the cobs, highly oxidized and reactive non-esterified linolenic acid is used to a greater extent than the less oxidized and less reactive non-esterified linoleic acid for the formation of grain cell membranes. This is indicated by a sharp decrease in the ratio of highly oxidized and highly reactive non-esterified linolenic acid to less oxidized and less reactive non-esterified linoleic acid.

The decrease in the content of non-esterified saturated fatty acids in the stem and leaves of control corn plants during growth is caused in particular by a decrease in the total content of saturated fatty acids with an even number of carbon atoms in the chain by 10% and an insignificant decrease in the level of acids with an odd number. On the other hand, the reduction in the content of non-esterified monounsaturated fatty acids in the green mass of corn plants of the control variant during growth occurs, in particular, due to a decrease in the level of oleic acid by 16%. In contrast, the content of palmitoleic acid practically does not decrease.

Table 2. The content of non-esterified fatty acids in the stem and leaves of corn in the phase of 8-9 leaves, $g \cdot 10^{-3}$/kg natural weight ($M \pm m$, $n = 3$)

Fatty acids and their code	Control	Experiment	
		$N_{60}P_{45}K_{45}$	Zeastimulin
Kaprynic, 10:0	0.24±0.01	0.20±0.01*	0.29±0.01*
Laurynic, 12:0	0.48±0.02	0.41±0.01	0.56±0.02
Miristynic, 14:0	0.79±0.03	0.68±0.02	0.90±0.02
Pentadekanic, 15:0	0.47±0.02	0.39±0.02	0.55±0.02
Palmytynic, 16:0	2.36±0.11	1.94±0.09*	2.73±0.09
Palmitooleinic, 16:1	0.25±0.01	0.20±0.01*	0.30±0.01*
Stearinic, 18:0	1.32±0.06	1.10±0.06	1.60±0.08
Oleinic, 18:1	2.72±0.13	2.25±0.12	3.15±0.10
Linolinic, 18:2	5.40±0.23	4.69±0.23	6.41±0.19*
Linolenic, 18:3	10.34±0.52	8.50±0.43	12.22±0.51
The total fatty acid content	24.37	20.36	28.71
including saturated	5.66	4.72	6.63
monounsaturated	2.97	2.45	3.45
polyunsaturated	15.74	13.19	18.63
linolenic/linoleic	1.91	1.81	1.91

Non-esterified forms of fatty acids perform several functions in plant organisms, of which special attention is paid to their role in forming plant resistance to adverse environmental conditions. However, there are practically no studies that explain the change in the content of non-esterified forms of fatty acids in the plant organism under the influence of exogenous factors. So, we investigated the nature of the change in the content of non-esterified forms of fatty acids in the grain of Zbruch hybrid corn during ripening and the effects of mineral fertilizers and the growth regulator Zeastimulin.

It was established that the content of non-esterified forms of saturated, monounsaturated, and polyunsaturated fatty acids in the stem and leaves of corn plants in all phases of intensive growth decreased more intensively compared to the control variant (Tables 2, 3, 4, and 5). This may indicate that mineral fertilization in the phase of intensive growth of maize plants intensifies the use of non-esterified fatty acids in the stem and leaves for synthesizing phospholipids, esterified cholesterol, and triacylglycerols, which are necessary for cell membranes and storage.

It should be noted that only in the phase of 8-9 leaves and panicle shedding under the influence of mineral fertilizer, compared to the control variant, the ratio of the content of highly oxidized and highly reactive non-esterified linolenic acid to less oxidized and less reactive non-esterified linoleic acid in the stem and leaves of corn plants is sharply reduced. It can be seen in the synthesis of biologically active substances that change the hormonal balance of plant tissues highly oxidized and highly reactive non-esterified linolenic acid is used to a greater extent than less oxidized and less reactive non-esterified linoleic acid.

Table 3. The content of non-esterified fatty acids in the stem and leaves of corn in the panic phase, g·10^{-3}/kg natural weight (M ± m, n = 3)

Fatty acids and their code	Control	Experiment	
		$N_{60}P_{45}K_{45}$	Zeastimulin
Kaprynic, 10:0	0.23±0.01	0.19±0.01*	0.28±0.01*
Laurynic, 12:0	0.47±0.02	0.39±0.02	0.55±0.02
Miristynic, 14:0	0.65±0.02	0.55±0.02*	0.76±0.02*
Pentadekanic, 15:0	0.46±0.02	0.38±0.02*	0.55±0.02*
Palmytynic, 16:0	2.26±0.11	1.86±0.09	2.68±0.11
Palmitooleinic, 16:1	0.24±0.01	0.19±0.01*	0.29±0.01*
Stearinic, 18:0	1.13±0.05	0.96±0.04	1.35±0.06
Oleinic, 18:1	2.35±0.10	2.28±0.35*	2.71±0.09
Linolinic, 18:2	5.48±0.19	4.75±0.20	6.22±0.18*
Linolenic, 18:3	10.13±0.55	8.52±0.37	12.08±0.52
The total fatty acid content	23.40	20.07	27.47
including saturated	5.20	4.33	6.17
monounsaturated	2.59	2.47	3.00
polyunsaturated	15.61	13.27	18.30
linolenic/linoleic	1.85	1.79	1.94

Zeastimulin, applied by us to treat corn plants according to the manufacturer's recommendations, caused the accumulation of non-esterified forms of saturated, monounsaturated, and polyunsaturated fatty acids in the stem and leaves of corn plants during their intensive growth, namely in the phase of 8-9 leaves, panicle shedding, flowering panicles and cobs (Tables 2, 3, 4 and 5).

The detected changes in the content of non-esterified forms of unsaturated fatty acids under Zeastimulin were accompanied by a gradual change in the ratio of highly oxidized and highly reactive non-esterified linolenic acid to less oxidized and less reactive non-esterified linoleic acid. In particular, it was established that during the phase of panicle shedding, panicle and cob flowering in the stem and leaves of corn plants, the ratio of highly oxidized and highly reactive non-esterified linolenic acid to less oxidized and less reactive non-esterified linoleic acid increases. In these phases of intense growth of corn plants, under the influence of the mentioned growth stimulator on the synthesis of biologically active substances that change the hormonal balance of plant tissues, highly oxidized and reactive non-esterified linolenic acid is used to a greater extent than less oxidized and less reactive non-esterified linoleic acid.

Table 4. The content of non-esterified fatty acids in the stem and leaves of corn in the panicle flowering phase, g·10^{-3}/kg of natural weight (M ± m, n = 3)

Fatty acids and their code	Control	Experiment	
		$N_{60}P_{45}K_{45}$	Zeastimulin
Kaprynic, 10:0	0.23±0.01	0.19±0.01*	0.28±0.01*
Laurynic, 12:0	0.46±0.02	0.39±0.01*	0.53±0.02*
Miristynic, 14:0	0.70±0.02	0.61±0.02*	0.79±0.02*
Pentadekanic, 15:0	0.45±0.02	0.37±0.02*	0.50±0.02
Palmytynic, 16:0	2.33±0.09	1.95±0.09*	2.70±0.09*
Palmitooleinic, 16:1	0.22±0.01	0.18±0.01*	0.27±0.01*
Stearinic, 18:0	1.16±0.07	0.92±0.49	1.39±0.05
Oleinic, 18:1	2.30±0.08	1.93±0.10*	2.67±0.10*
Linolinic, 18:2	5.38±0.012	4.82±0.19	5.92±0.15*
Linolenic, 18:3	9.93±0.24	8.98±0.21*	11.10±0.42
The total fatty acid content	23.16	20.34	26.15
including saturated	5.33	4.43	6.19
monounsaturated	2.52	2.11	2.94
polyunsaturated	15.31	13.8	17.02
linolenic/linoleic	1.85	1.86	1.88

Thus, during the intensive growth of the green mass of corn plants in the stem and leaves, the content of non-esterified forms of saturated, monounsaturated fatty acids, and polyunsaturated fatty acids decreases. In general, this may be due to their greater use for cells' energy needs, the synthesis of lipids, phytohormones, and other biologically active substances.

The content of non-esterified forms of fatty acids in the stem and leaves of corn plants in the intensive growth phase generally decreases under the influence of mineral fertilizers and increases under the influence of Zeastimulin. This indicates differences in the biochemical action of fertilizer and growth stimulator on the plant cell. As you know, fats act on plant cells due to various amino acid and fatty acid metabolites, and growth stimulants are mainly due to synthesizing biologically active substances. There are data in the literature that as a result of the oxidation of fatty acid components in plant cells, such biologically active substances as oxylipins are formed. The latter has a wide range of regulatory influences and acts on the plant cell in minimal concentrations.

In the final phases of intensive growth (ejection of panicles, flowering of panicles and cobs) in the stem and leaves of corn plants under the action of a growth stimulator, an increase in the ratio of the content of the non-esterified form of linolenic acid to the non-esterified form of linolenic acid was noted, which indicates the possibility of the effect of the active components of Zeastimulin on lipids, in particular fatty acids, plant cell components. Polyunsaturated fatty acid linolenic acid has a wider range of biological effects on plant cells than polyunsaturated fatty acid linoleic acid.

Table 5. The content of non-esterified fatty acids in the stem and leaves of corn in the cob flowering phase, $g \cdot 10^{-3}/kg$ of natural weight ($M \pm m$, $n = 3$)

Fatty acids and their code	Control	Experiment	
		$N_{60}P_{45}K_{45}$	Zeastimulin
	0.20±0.01	0.17±0.01*	0.23±0.01*
Kaprynic, 10:0	0.46±0.02	0.39±0.02*	0.53±0.02*
Laurynic, 12:0	0.62±0.02	0.54±0.00	0.72±0.02*
Miristynic, 14:0	0.45±0.02	0.38±0.01*	0.53±0.02*
Pentadekanic, 15:0	1.78±0.07	1.42±0.06*	2.04±0.05*
Palmytynic, 16:0	0.21±0.01	0.18±0.01*	0.25±0.01*
Palmitooleinic, 16:1	1.24±0.01	0.95±0.06*	1.52±0.05
Stearinic, 18:0	2.40±0.01	2.04±0.08	2.79±0.09
Oleinic, 18:1	4.76±0.01	4.21±0.12*	5.33±0.12*
Linolinic, 18:2	8.32±0.18	7.53±0.21*	8.99±0.16*
Linolenic, 18:3	20.44	17.81	22.93
The total fatty acid content	4.75	3.85	5.57
including saturated	2.61	2.22	3.04
monounsaturated	13.08	11.74	14.32
polyunsaturated	0.57	0.56	0.59

It was found that the increase in the functional activity of the photosynthetic apparatus of corn plants in the phase of intensive growth is accompanied by an increase in the concentration of esterified fatty acids and the ratio of the content of the more valuable polyunsaturated fatty acid linoleic to the less valuable polyunsaturated fatty acid linoleic in the stem and leaves. Under the influence of mineral fertilizer and growth regulator Zeastimulin in the intensive growth phase of corn plants, esterified fatty acids accumulate, and the ratio of polyunsaturated linolenic fatty acid to polyunsaturated linoleic fatty acid in the stem and leaves increases. Esterified fatty acids in the composition of phospholipids in the green mass of corn primarily form cell membranes (Bates et al., 2013). Secondly, esterified fatty acids in the composition of triacylglycerols are deposited in the stem, leaves, and grain of corn, thereby creating their energy and biological value (Wang et al., 2020). The main poly saturated fatty acids of plant grains - linoleic and linolenic - play an essential role in the complete nutrition of humans and animals (Taran et al., 2006).

Given the importance of the functions performed by fatty acids in the plant organism in the conditions of field research, we studied the peculiarities of changes in their content in the green mass of corn plants. We established that during the control plots' intensive growth of corn plants, esterified fatty acids accumulated in their green mass (stems and leaves) (Tables 6, 7, and 8). Furthermore, their content increases due to saturated, monounsaturated, and polyunsaturated fatty acids.

The above is possibly related to the fact that in plant tissues from acetate and propionate, under the influence of synthase enzymes, saturated fatty acids are first synthesized, respectively, with an even (capric, lauric, myristic, palmitic, and stearic) and odd (pentadecane) number of carbon atoms in the chain. Then monounsaturated fatty acids are formed from saturated fatty acids under a specific desaturate enzyme (from palmitic to palmitooleic, and from stearic to oleic) (Taran et al., 2006). Only in plant tissues, under the influence of other specific desaturase enzymes, linoleic polyunsaturated fatty acid, which is valuable for plants, humans, and animals, is first formed from the monounsaturated fatty acid oleic. Then the latter forms an even more valuable unsaturated fatty acid, linolenic acid (Taran et al., 2006; Qin et al., 2013).

It should be noted that the increase in the concentration of unsaturated fatty acids in the stem and leaves of control corn plants in the phase of panicle ejection and flowering, compared to the phase of 8-9 leaves, is observed mainly due to polyunsaturated fatty acids linoleic and linolenic. A gradual growth in the ratio of linolenic acid content to linoleic acid accompanies this. This increases the functional activity of plant tissues.

Compared to the control, applying soil fertilizer significantly raised the intensity of accumulation of saturated, monounsaturated, and polyunsaturated fatty acids of total lipids in the stem and leaves of corn during intensive growth. It is worth noting the increase in the ratio of the content of the very valuable polyunsaturated fatty acid linolenic acid to the less valuable linoleic acid in the stems and leaves of corn plants under the action of fertilization, the nature of which is very pronounced in the phase of flowering and, especially, the shedding of the panicle.

It was investigated that the peculiarities of the dynamics of the accumulation of esterified fatty acids in the stem and leaves of corn during the period of intensive growth under the influence of the growth regulator Zeastimulin did not differ from those described by us under the action of fertilizers, their growth occurred equally due to saturated, monounsaturated and polyunsaturated fatty acids. At the same time, the ratio of the content of polyunsaturated fatty acid linolenic acid to polyunsaturated fatty acid linoleic acid gradually increased.

Table 6. The content of esterified fatty acids in the stem and leaves of corn in the phase of 8-9 leaves, $g \cdot 10^{-3}$/kg of natural weight (M ± m, n = 3)

Fatty acids and their code	Control	Experiment	
		$N_{60}P_{45}K_{45}$	Zeastimulin
Kaprynic, 10:0	2.4±0.11	2.9±0.14*	3.0±0.11*
Laurynic, 12:0	4.8±0.20	5.9±0.29*	6.0±0.32*
Miristynic, 14:0	7.2±0.32	8.5±0.32*	8.8±0.35*
Pentadekanic, 15:0	4.8±0.23	5.7±0.23	5.8±0.26*
Palmytynic, 16:0	24.2±1.24	29.9±1.56*	30.4±1.66*
Palmitooleinic, 16:1	2.4±0.145	3.2±0.17*	3.1±0.17*
Stearinic, 18:0	12.2±0.61	15.5±0.72*	15.5±0.81*
Oleinic, 18:1	26.8±1.57	32.7±1.56	34.0±1.68*
Linolinic, 18:2	55.3±2.48	65.7±2.54*	67.0±2.77*
Linolenic, 18:3	102.5±4.69	124.3±6.67	125.2±6.54*
The total fatty acid content	242.6	294.3	298.8
including saturated	55.6	68.4	69.5
monounsaturated	29.2	35.9	37.1
polyunsaturated	157.8	190.0	192.2

Table 7. Thecontentofesterifiedfattyacidsinthestemandleavesofcorninthepanicphase, g·10^{-3}/kgofnaturalweight ($M \pm m$, n = 3)

Fatty acids and their code	Control	Experiment	
		$N_{60}P_{45}K_{45}$	Zeastimulin
Kaprynic, 10:0	2.6±0.17	3.4±0.17*	3.5±0.21*
Laurynic, 12:0	5.2±0.26	6.3±0.26*	6.4±0.20*
Miristynic, 14:0	7.8±0.35	9.2±0.32*	9.4±0.29*
Pentadekanic, 15:0	5.1±0.23	6.0±0.20*	6.2±0.20*
Palmytynic, 16:0	26.2±1.24	32.3±1.68*	33.7±1.91*
Palmitooleinic, 16:1	2.6±0.145	3.4±0.17*	3.4±0.20*
Stearinic, 18:0	12.6±0.61	15.1±0.66*	15.5±0.65*
Oleinic, 18:1	26,4±1,38	31.8±1.34*	32.8±1.65*
Linolinic, 18:2	60.1±2.19	70.4±2.63*	71.7±2.46*
Linolenic, 18:3	111.7±5.05	136.6±7.28*	138.5±7.28*
The total fatty acid content	260.3	314.5	321.1
including saturated	59.5	72.3	74.7
monounsaturated	29.0	35.2	36.2
polyunsaturated	171.8	207.0	210.2

Table 8. The content of esterified fatty acids in the stem and leaves of corn in the panicle flowering phase, g·10^{-3}/kg of natural weight ($M \pm m$, n = 3)

Fatty acids and their code	Control	Experiment	
		$N_{60}P_{45}K_{45}$	Zeastimulin
Kaprynic, 10:0	2.9±0.17	3.6±0.17*	3.8±0.20*
Laurynic, 12:0	5.8±0.26	7.0±0.23*	7.2±0.23*
Miristynic, 14:0	8.7±0.34	10.2±0.34*	10.4±0.40*
Pentadekanic, 15:0	5.6±0.20	6.6±0.23*	6.7±0.23*
Palmytynic, 16:0	29.4±1.76	36.1±1.56*	36.8±1.6*
Palmitooleinic, 16:1	2.8±0.17	3.5±0.14*	3.7±0.17*
Stearinic, 18:0	14.5±0.72	17.4±0.81	17.6±0.75*
Oleinic, 18:1	28.4±1.07	34.1±1.50*	34.7±1.51*
Linolinic, 18:2	66.7±2.68	78.8±3.29*	80.4±3.44*
Linolenic, 18:3	124.5±6.61	150.6±6.90	152.6±6.75*
The total fatty acid content	289.3	347.9	353.9
including saturated	66.9	80.9	82.5
monounsaturated	31.2	37.6	38.4
polyunsaturated	191.2	229.4	233.0

In the ontogenesis of plants, in each period of their development, the course and direction of the main metabolic processes are determined by the actual requests of the plant organism (Kobyletska, 2020). During the transition of a plant from the juvenile period of development, which is characterized by an intensive accumulation of vegetative mass, to the generative one, an ontogenetic restructuring of metabolism takes place, due to the appearance and formation of generative organs, which in this period become integration centers and the main acceptors of plastic and energy resources of the plant (Miyazaki et al., 2013; Perlikowski et al., 2016).

We found that when corn plants transition to the generative phase of development when grain formation occurs, the content of esterified fatty acids in the stem and leaves decreases (Table 9). It is shown that the decrease in their content under these conditions occurs mainly due to saturated and polyunsaturated fatty acids. At the same time, the ratio of the content of the precious polyunsaturated fatty acid linolenic acid to the less valuable polyunsaturated fatty acid linoleic acid also begins to decrease in the stalk and leaves of corn due to the greater use of highly oxidized and highly active linolenic acid for synthesizing biologically active derivatives.

Plant growth, as an integral process of vital activity, is closely related to its metabolism, in particular, to protein and lipid exchanges, thanks to which the genetic information of deoxyribonucleic acids is implemented in the corresponding structural and enzymatic proteins that regulate the functional activity of cells (Yang et al., 2013).

Table 9. The content of esterified fatty acids in the stem and leaves of corn in the cob flowering phase, $g \cdot 10^{-3}$/kg of natural weight (M ± m, n = 3)

Fatty acids and their code	Control	Experiment	
		$N_{60}P_{45}K_{45}$	Zeastimulin
Kaprynic, 10:0	2.5±0.11	3.2±0.14*	3.1±0.17*
Laurynic, 12:0	5.8±0.23	6.9±0.26*	6.8±0.26*
Miristynic, 14:0	7.8±0.32	9.4±0.32*	9.2±0.32*
Pentadekanic, 15:0	5.6±0.23	6.9±0.26*	6.7±0.23*
Palmytynic, 16:0	26.2±1.36	33.0±1.47*	32.1±1.56*
Palmitooleinic, 16:1	2.6±0.17	3.4±0.09*	3.3±0.11*
Stearinic, 18:0	15.4±0.62	18.6±0.69*	18.1±0.72*
Oleinic, 18:1	31.3±1.50	38.6±1.43*	38.0±1.33*
Linolinic, 18:2	59.4±2.81	71.9±2.11*	70.2±2.37*
Linolenic, 18:3	104.1±5.17	127.3±6.00*	125.4±5.92
The total fatty acid content	260.7	319.2	312.9
including saturated	63.3	78.0	76.0
monounsaturated	33.9	42.0	41.3
polyunsaturated	163.5	199.2	195.6

The basis of plant growth is correlational relationships, both between individual organs and between individual processes. The visible results of plant growth are, in particular, a change in size and biomass accumulation. This complex physiological process directly or indirectly depends on many exo- and endogenous factors, often interconnected (Zhu et al., 2014; Guidi et al., 2019).
The peculiarity of the growth of corn plants is that, unlike other cereals, they can absorb elements of mineral nutrition before the grain is fully ripe, and the central mass of organic matter is accumulated after the panicles are thrown out and during the maturation of the reproductive organs (Kramarov, 2003; Deeva, 2008).
Plant height is an essential biometric indicator of growth and development and, therefore, productivity. We have shown in field studies that the intensity of growth of hybrid Zbruch corn plants varied depending on their morphological and individual characteristics and depended on the vegetation phase, the action of fertilizers, and the growth regulator Zeastimulin (Table 10).

Table 10. TheheightofthestemofcornplantsoftheZbruchhybridduringtheperiodofintensivegrowth, see (M ± m, n = 10)

Phases vegetation	Control	Experiment	
		$N_{60}P_{45}K_{45}$	Zeastimulin
8-9 leaves	65.8±2.19	73.0±1.12	71.9±2.40
Ejection of the panicle	197.1±2.57	207.5±2.29	210.5±2.75*
Flowering cabbage	199.3±2.98	221.0±2.64*	223.0±2.87*

As the above table shows, corn plants' growth intensity naturally increases with age, reaching maximum values in the panicle ejection and flowering phase. Under the influence of soil fertilizer, there is an increased rate of corn plants in the tillering phase by 10% compared to the control, while under the action of the growth regulator Zeastimulin - by 9%. In the following phases of vegetation, growth rates of corn plants were found to increase by 9-12% under Zeastimulin, while under the action of soil fertilizer - by 5-10%.
To understand the features of productivity formation processes, in addition to linear growth intensity parameters, quantitative indicators of plant growth and development are used, one of which is the dynamics of the accumulation of raw mass or dry matter during the growing season. That is why, to evaluate the influence of the Zeastimulin regulator and fertilizer, we studied the change in the raw mass of corn plants (Table 11).

Table 11. Accumulation of raw mass by corn plants during the period of intensive growth, g/plant (M ± m, n = 10)

Phases growth	Control	Experiment	
		$N_{60}P_{45}K_{45}$	Zeastimulin
8-9 leaves	298.8±19.00	340.1±6.80*	341.3±7.30*
Ejection of the panicle	670.0±54.85	756.3±12.90	736.4±11.15*
Flowering cobs	1285.5±103.50	1340.4±15.00	1315.7±14.65

As you can see, the intensity of accumulation of raw mass by corn plants naturally increases with age, reaching maximum values during panicle shedding and cob flowering. Under the influence of soil fertilizer, there is an increase in the rate of accumulation of raw mass by corn plants in the tilling phase by 10% relative to the control, while under the action of the growth regulator Zeastimulin - by 9%. In the following phases of the growing season, an increase in the accumulation rate of raw mass by corn plants was found under the action of Zeastimulin by 9-12%, while under the action of soil fertilizer - by 5-10%.

The marked increase in the intensity of growth of corn plants under the influence of fertilizer can be explained, in particular, by their physiological features since they, as representatives of the C-4 type of photosynthesis of trophic origin, have a high genetic potential for growth, which is more fully realized under conditions of optimal and intensive root nutrition, which is especially relevant for new varieties and hybrids (Ponomarenko, 2003).

The activation of the accumulation of raw mass by corn plants under the influence of the growth regulator Zeastimulin is explained by its ability, similar to other active analogs of phytohormones, to impact growth by replenishing the endogenous pool of phytohormones, thereby changing the phytohormonal balance of the plant and activating, in particular, the processes of cell division (Storozhenko, 2004; Cook et al., 2021). As a result of the formation of a significant number of cells, stretching and linear maturation of the plant organism is accelerated.

As we can see from the given data, corn plants quite intensively accumulate raw mass in all investigated phases of intensive growth. The most optimal of the investigated factors was the effect of soil fertilizer, under the influence of which an increase in the raw mass of 1 corn plant by 50-69 g was observed, while under the influence of Zeastimulin this effect was less pronounced - an rise of 30-40 g was shown.

In general, the active growth of corn plants in height and their accumulation of raw mass under the action of plant growth regulators can be activated, in particular, due to the acceleration of cell growth by stretching in the longitudinal and isodiametric directions, the increase in the permeability of cell membranes, which is activated explicitly by auxin-like components of biologically active compounds (Miyazaki et al., 2013). The increase in the intensity of the growth of corn plants due to the action of mineral fertilizers is due to the additional optimization of the soil nutrition of these plants associated with this better supply of nutrients and the opportunity to realize the productive potential (Ali, 2021) more fully.

CONCLUSIONS

The functional activity of the photosynthetic apparatus in the leaves of corn plants increases with their age and depends on soil fertilization and the influence of growth regulators. This is confirmed by the fact that the leaves of corn plants accumulate photosynthetic pigments during intensive growth and under the influence of mineral fertilizers and the growth regulator Zeastimulin.

The increase in the functional activity of the photosynthetic apparatus of corn plants during the period of intensive growth is accompanied by a slight and gradual decrease in the concentration of non-esterified fatty acids but an increase of esterified ones, in the stem and leaves, from the phase of 8-9 leaves to the phase of panicle flowering. During the cob flowering phase, the concentration of non-esterified and esterified fatty acids in the stem and leaves of corn sharply decreases. In the same phase, the ratio of the content of the more valuable linoleic fatty acid to the less valuable linoleic fatty acid also sharply decreases in the stalk and leaves of corn.

The general pattern of changes in the concentration of non-esterified and esterified fatty acids and the ratio of the content of the more valuable linoleic fatty acid to the less valuable linoleic fatty acid in the stem and leaves in all phases of the vegetation phase of corn plants under the influence of mineral fertilizer and the growth regulator Zeastimulin resembles the changes in the control version. But changes in the concentration of non-esterified fatty acids in the stem and leaves of corn plants under the influence of mineral fertilizers occur at a lower level and under the influence of the growth regulator Zeastimulin - at a higher level. Only in the flowering phase of cobs under the influence of mineral fertilizer and growth regulator Zeastimulin in the stem and leaves of corn plants does the concentration of non-esterified and esterified fatty acids increase without changing the ratio of linoleic acid to linoleic acid.

The height of corn plants and the intensity of their accumulation of raw mass increases with age and reaches maximum values during panicle shedding and cob flowering. Under the influence of soil fertilizer and the growth regulator Zeastimulin, the rate of accumulation of raw mass by corn plants increases.

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TECHNOLOGICAL SOLUTIONS FOR COMMON SUNFLOWER (*Heliantus annuus*) GROWING IN A CHANGING CLIMATE CONDITION

Atanas SEVOV[1], Veska GEORGIEVA[2]

[1]Agricultural University of Plovdiv, 12 Mendeleev Blvd, Plovdiv, Bulgaria
[2]National Institute of Meteorology and Hydrology, Sofia, Bulgaria

Corresponding author email: veska.georgieva@meteo.bg

Abstract

Climatic changes in recent years have adversely affected the development of a number of crops. Especially the spring ones suffer, since the development phases in which the yield is formed take place during the periods most strongly influenced by climate change. In the present study, the different climate components during the period 1991-2020 that most strongly influence sunflower development are characterized. It was established that the unfavorable trends of changes in agrometeorological conditions in the studied area are related to a decrease in summer precipitation and an increase in temperatures during the same period. As a result, the last two months of the sunflower growing season are in a moisture deficit. Temperatures in the region during the studied period have increased, with positive deviations in all months, except for December. No differences were recorded in the dates of the transition of the air temperature through 5ºC. During the researched period, they increased by 167ºC compared to the previous one. During the studied period, precipitation in summer decreased at the expense of its increase in autumn, compared to the previous one.

Key words: *climate change, climate conditions, sunflower.*

INTRODUCTION

In recent years, sunflower yields in Bulgaria have been increasing, but to be profitable, they must exceed 250 kg (Mikova, 2020). Environmental conditions determine 65-80% of plant productivity (Hogenboom, 2000), but the analysis of the agroclimatic potential of the region and the adaptation of the cultivation technology to the peculiarities of the agrometeorological conditions is the basis of good crop yields.

In recent years, we have witnessed unfavorable trends in changes in the main meteorological elements, as well as an increase in extreme weather events. This puts sunflower growing in some traditional areas of crop production at risk.

Since the beginning of the 21st century, we have witnessed annual weather and climate records - globally and nationally. The frequency of extreme weather events increases every year. The World Meteorological Organization (WMO) marks the fifth warmest year since the beginning of the 21st century.

The climate in Bulgaria changed not only to warmer but also to drier at the end of the 20^{s} of our century. In the last decade, however, total rainfall has increased, but heavy rainfall is more damaging than irrigating. An example of this is the rainiest year for the entire period 1988-2016 - 2014 when the average annual amount of rainfall was 1013 mm for the areas up to 800 m above sea level, which is more than previously reached a maximum of 924 mm in 2005 (Marinova et al., 2017). The rainiest months are September and October. In 2014, were measured extreme amounts of rainfall in 24 hours between April and October. The largest value of 245 mm (Burgas region) ranks 2014 among the seven years in the 1988-2014 period with extreme daily rainfall above 220 mm.

The assessment of agrometeorological conditions in Bulgaria during the period 1986-2015 in Bulgaria based on 55 meteorological stations shows an average monthly air temperature increase in the months from January to September for all stations of 0.5º-1.5ºC. The biggest deviations are observed in January, July and August.

The significant increase of the yearly rainfall sums is observed in Northeastern part of the country - Razgrad, Shumen and Varna in about 10-18%. Insignificant decrease is detected in part of stations in Central and Western Bulgaria (Georgieva et al., 2022).

The indicated trends of changes in temperature and humidity conditions are unfavorable for cultivating sunflowers, especially in Central South Bulgaria. Therefore, the present study aims to investigate the agro-climatic potential and the changing trends of the main agro-climatic indicators in this area and to indicate technological solutions to increase the profitability of sunflower cultivation.

MATERIALS AND METHODS

Study area

The study area covers Central South Bulgaria. According to the climatic division of Bulgaria, it falls into two climatic regions and sub-regions and 3 climatic regions (Table 1.). Most of the territory of the studied region falls into the transitional-continental climate subregion of the European-continental climate region and the regions south of Haskovo in the South Bulgarian climate subregion of the Continental-Mediterranean climate region (Sabev et al., 1959).

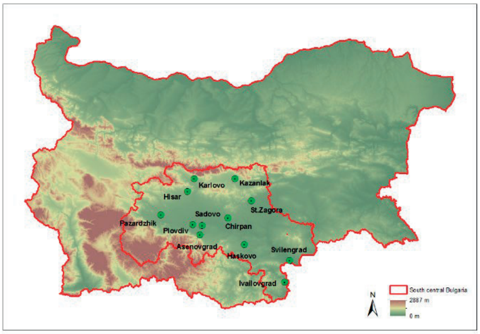

Figure 1. Location of the stations

The climatic region of Eastern Central Bulgaria is characterized by relatively mild winters. The average air temperature in January is around and above 0°C, with relatively frequent warming under the influence of Mediterranean cyclones. Rainfall in winter is between 100-150 mm. Due to the milder winter, spring starts early, and the temperature remains above 5°C at the end of February beginning of March. Summer in the area is hot. Rainfall conditions in the area are continental. In the Northern part of the region, summer rainfall exceeds winter rainfall by 10% of the annual amount. In the eastern and southern parts of the region, the seasonal amounts level off and even the maximum passes into autumn or spring.

Table 1. Distribution of the studied stations by climatic regions

Climatic region	Stations
1. Climatic region of Eastern Central Bulgaria	St. Zagora, Chirpan, Plovdiv, Sadovo, Asenovgrad, Pazardzhik, Hisar
2. Climatic region of the Eastern Sub-Balkan fields	Karlovo and Kazanlak
3. Climatic region of the Eastern Rhodope river valleys	Haskovo, Svilengrad, Ivaylovgrad

Winter in the climatic region of the Eastern Sub-Balkan Fields is mild, but due to the hollow character of the terrain, the absolute minimum temperatures reach lower values. The basin-like nature of the terrain also affects the frost regime. Summer is moderately warm. The seasonal distribution of rainfall has a continental character. Summer rainfall amounts are the largest, and winter rainfall amounts are the smallest. The difference reaches 10-15% of the annual amount. In the second half of summer, prolonged droughts often occur.

In the climate of the Eastern Rhodope river valleys, some of the largest rainfalls were reported from the lowland part of the country during the autumn-winter period. Winter in the area is mild. Everywhere in the region, the average January temperature is positive. The spring here is one of the warmest in the country. Summer is hot and dry, with the most rainfall in June. Then a long period with no rainfall begins, well expressed in late summer and early autumn. Autumn is warmer than spring. Due to the high-temperature sums that accumulate during the period with temperatures higher than 5 and 10°C, the region has some of the largest thermal resources in the country. The seasonal distribution of rainfall in the region with an autumn-winter maximum and a summer minimum.

Agrometeorological conditions for growing sunflowers in Bulgaria

The biological minimum of the culture is 5°C, but optimal conditions for germination are obtained at temperatures of 10-12°C. Young plants tolerate short-term colds up to - 5°C. The optimal temperature for culture development during critical phases is 20-25°C. Temperatures above 30°C already have a stagnant effect. The temperature sum for ripening the seeds under our conditions is 2500°C. The sunflower is relatively drought-resistant, as it satisfies its needs for moisture from the deeper soil layers. A critical period of moisture is the period from budding to flowering. From the inflorescence formation to the flowering phase, plants use about 45% of the water they need, and the maximum water consumption per day reaches 5-6 m^3/ha. From flowering to full maturity, plants use an average of 35% of all required water.

Data and methods

The study period is 1991-2020, and the reference period is 1961-1990. Daily temperature and rainfall data were used at 11 stations, Figure 1. To estimate the moisture conditions, we use the Balance of atmospheric humidity:

$$BAH = \frac{ETo}{\sum r}.,$$

ETo is the potential evapotranspiration calculated by the Penman-Monteith equation using CROPWAT8, $\sum r$ is the sum of rainfall.

In order to detect and estimate trends in the 30-year time series of the meteorological factors air temperature, rainfall totals, VPD totals, as well as of AI_{DM} and ETo, Mann-Kendall test was applied. Theoretically, the data values x_i of the 40-year time series were assumed to obey the model

$$x_i = f(t_i) + \varepsilon_i$$

where $f(t_i)$ is a continuous monotonic increasing (or decreasing) function of time, and the residuals ε_i can be assumed to be with zero mean, i.e. the variance of the distribution is constant in time.

By Mann-Kendall test we tested the null hypothesis of no trend, H_o, against the alternative hypothesis, H_l, where there is an increasing or decreasing monotonic trend. S-statistics and the normal approximation (Z statistics) were exploited.

$$S = \sum_{k=1}^{n-1} \sum_{j=k+1}^{n} sgn(x_j - x_k)$$

where x_j and x_k are the annual values in years j and k, j>k, respectively and:

$$sgn(x_j - x_k) = \begin{cases} 1 & if\ (x_j - x_{k)} > 0 \\ 0 & if\ (x_j - x_{k)} = 0 \\ -1 & if\ (x_j - x_{k)} < 0 \end{cases}$$

The variance of S was computed by the following equation which took into account that ties may be present:

$$VAR(S) = \frac{1}{18}\left[n(n-1)(2n+5) - \sum_{p=1}^{q} t_p(t_p - 1)(2t_p + 5)\right]$$

where t_p - number of data values in the p^{th} group; q - number of tied groups; n – number of the consequent data in the time series.

The presence of a statistically significant trend was evaluated using the Z value, which was calculated by using S and $VAR(S)$:

$$Z = \begin{cases} \frac{S-1}{\sqrt{VAR(S)}} & if\ S > 0 \\ 0 & if\ S = 0 \\ \frac{S+1}{\sqrt{VAR(S)}} & if\ S < 0 \end{cases}$$

The condition for upward trend is if Z>0 and vice versa. To test for either an upward or downward monotone trend (a two-tailed test) at a level of significance, H_0 was rejected if $|Z| > Z_{1-\alpha/2}$, where $Z_{1-\alpha/2}$ was obtained from the standard normal cumulative distribution tables. The tested significance levels were α = 0.001, α = 0.01, α = 0.05 and α = 0.1.

RESULTS AND DISCUSSIONS

The average monthly air temperature during the studied period 1991-2020 is positive in all months (Figure 2). In the coldest month - January, the average monthly air temperatures exceed 2°C in the southernmost regions. Temperatures in central and western regions of the studied area - Pazardzhik, Sadovo and Kazanlak from the Sub-Balkan fields - are below 1°C. The lowest January temperature was recorded in Chirpan. In the warmest month of July, the average monthly values are equal to those in August (St. Zagora, Haskovo, Hisar and Ivaylovgrad), and in some stations, even August temperatures are higher than those in July (Asenovgrad, Karlovo and Chirpan).

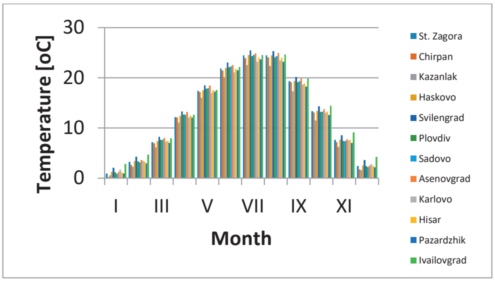

Figure 2. Average multi-annual monthly air temperatures during the period 1991-2020

The deviations of average monthly temperatures during the studied period compared to the reference (1961-1990) show an increase from January to November and a decrease in most stations in December, reaching 0.6°C (Figure 3). Exceptions are Asenovgrad and Ivaylovgrad stations. In January and February, the increase fluctuates between 0.2°C and 0.7°C, and in March, it exceeds 1°C in Svilengrad, Sadovo and Hisar. An increase of 1 or more than 1°C was also reported in May in Svilengrad and Hisar. The increase in average monthly air temperatures is greatest in July and August. In July, only in Karlovo and Ivaylovgrad it is less than 1°C. In August, the increase exceeded 2°C in almost all stations except Sadovo, Pazardzhik and Ivaylovgrad.

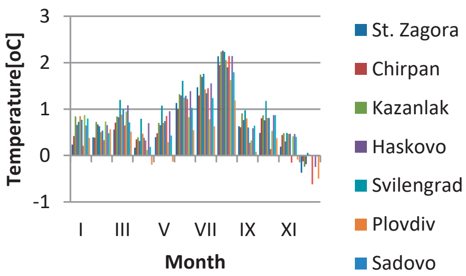

Figure 3. Deviation of the average monthly temperature for the period 1991-2020 compared to 1961-1990

The rainfall regime very clearly distinguishes the two climatic regions. Ivaylovgrad, Svilengrad and Haskovo stations have an autumn-winter maximum and a summer minimum, with the highest rainfall values in autumn and winter in Ivailovgrad. In the summer, the lowest rainfall amounts were recorded there - in July - 28.4 mm, and in August - 19.6 mm. In the remaining stations, the rainfall regime is summer, with maximum values exceeding 80 mm in Karlovo (Figure 4).

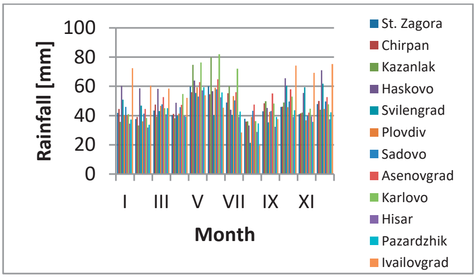

Figure 4. Average multi-annual monthly rainfall during the period 1991-2020

The most significant deviations of rainfall during the studied period compared to the reference period, exceeding 10%, were noted in February, March, September and October - an increase, and in April, June, August, November and December - a decrease. In July, a decrease was reported only at the Hisar station, and in Asenovgrad and Svilengrad, the rainfall increased significantly by 13 and 33%, respectively (Figure 5).

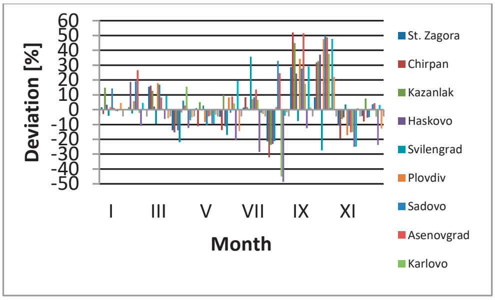

Figure 5. Deviations of monthly rainfall totals for 1991-2020 compared to the reference period 1961-1990

Temperature conditions for the growth and development of sunflowers in Central South Bulgaria

Under these weather conditions during the period 1991-2020, the permanent transition of the daily air temperature above 5°C in the Central South Bulgaria region is observed in the second decade of February, except for the Chirpan and Karlovo regions, where this happens later (Figure 6).

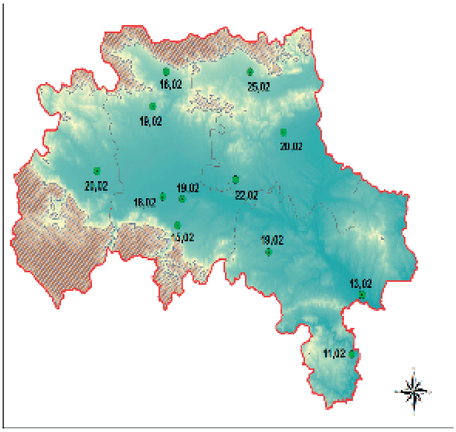

Figure 6. The average date of permanent transition of air temperature above 5°

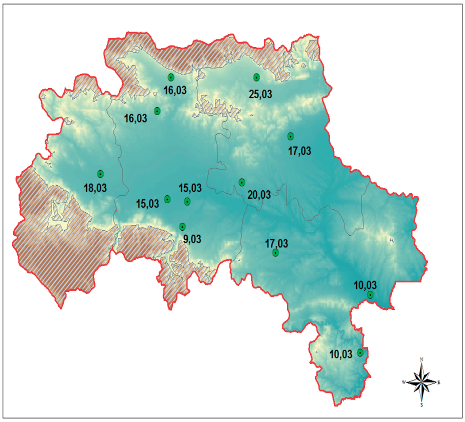

Figure 8. The average date of permanent transition of air temperature above 10° with a probability of 75%

The increase in average daily temperatures above 10°C with a probability of 50% takes place during the last ten days of March for most of the region and during the second for the southern parts (Figure 7). With a probability of 75% or once every 4 years, the date of the transition through 10ºC is observed in the southernmost regions at the end of the first decade of March, in the second decade in the rest, except for Kazanlak, where it happens on March 25 (Figure 8).

During the period with temperatures higher than 10°C in the central and southern regions of Central South Bulgaria, temperatures sum between 4200°C and 4430°C accumulate (Figure 9), while in the northern regions, they are close to 4000°C.

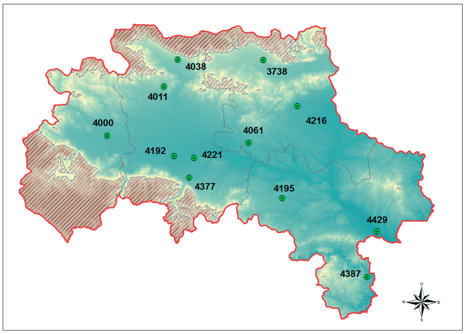

Figure 9. A sum of active temperatures during the period with temperatures higher than 10°C

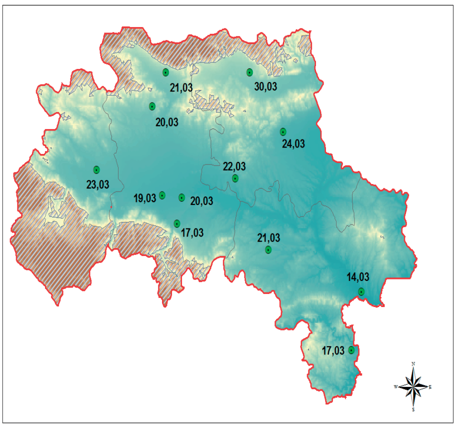

Figure 7. The average date of permanent transition of air temperature above 10ºC with a probability of 50%

Humidification conditions in Central South Bulgaria for sunflower cultivation

The amount of rainfall during the sunflower growing season - April-July exceeds 200 mm, except for the Svilengrad, Ivaylovgrad, Hisar and Pazardzhik stations (Figure 10). At the same time, potential evapotranspiration exceeds twice the amount of rainfall and reaches values of 480-600 mm/ºC (Figure 11). The amount of rainfall during the April-July sunflower growing season is between 33 and 53% of the amount of potential evapotranspiration. In the conditions of Central South Bulgaria, the growing season of the sunflower takes place in conditions of moisture deficiency. This ratio is unfavorable in Svilengrad, Ivaylovgrad and Pazardzhik 33-35%.

The study of the changing trends of the average multi-annual amount of rainfall for the studied stations during the period 1991-2020 shows that in most stations, there are no proven changing trends, except for the Plovdiv region, where all three stations show an increase in 90 and 95% confidence interval (Table 2).

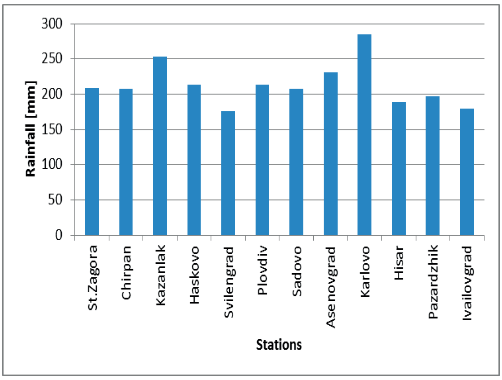

Figure 10. Average multi-annual amount of rainfall in [mm] April-July

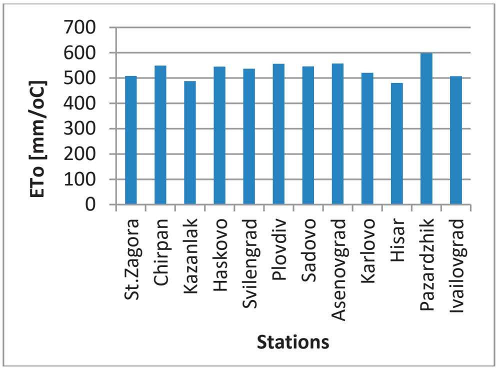

Figure 11. Average multi-annual sum of ETo [mm/°C] during April-July period

Table 2. Evaluation of the significance of the changing trend in rainfall [mm] VI-VII during the studied period. The tested significance levels were α = 0.001, α = 0.01, α = 0.05 and α = 0.1

Time series	First year	Last Year	n	Test S	Test Z	Signific.
St. Zagora	*1991*	*2020*	*30*		0.79	
Chirpan	*1991*	*2020*	*30*		0.09	
Kazanlak	*1991*	*2020*	*30*		0.82	
Haskovo	*1991*	*2020*	*30*		0.82	
Svilengrad	*1991*	*2020*	*30*		0.57	
Plovdiv	*1991*	*2020*	*30*		2.03	*
Sadovo	*1991*	*2020*	*30*		1.93	+
Asenovgrad	*1991*	*2020*	*30*		1.96	*
Karlovo	*1991*	*2020*	*30*		1.61	
Hisar	*1991*	*2020*	*30*		0.50	
Pazardzhik	*1991*	*2020*	*30*		1.07	
Ivailovgrad	*1991*	*2020*	*30*		1.43	

The same study at ETo shows an increase in the 90 and 95% confidence interval in Kazanlak and Chirpan and a significant increase in Ivaylovgrad in the 99.9% interval in Ivaylovgrad (Table 3).

Table 3. Evaluation of the significance of the trend of the change in the amount of ETo [mm/°C] VI-VII during the studied period

Time series	First year	Last Year	n	Test S	Test Z	Signific.
St. Zagora	*1991*	*2020*	*30*		0.20	
Chirpan	*1991*	*2020*	*30*		2.03	*
kazanlak	*1991*	*2020*	*30*		1.89	+
Haskovo	*1991*	*2020*	*30*		-0.25	
Svilengrad	*1991*	*2020*	*30*		-0.98	
Plovdiv	*1991*	*2020*	*30*		-1.03	
Sadovo	*1991*	*2020*	*30*		-0.82	
Asenovgrad	*1991*	*2020*	*30*		0.00	
Karlovo	*1991*	*2020*	*30*		-0.64	
Hisar	*1991*	*2020*	*30*		0.45	
Pazardzhik	*1991*	*2020*	*30*		-0.86	
Ivailovgrad	*1991*	*2020*	*30*		3.66	***

The critical period for the formation of the sunflower yield is the formation of an inflorescence - flowering. The amount of rainfall during this period did not exceed 100 mm, except for Karlovo and Asenovgrad (Figure 13). During the period of vegetation , potential evapotranspiration exceeds three times the rainfall and fluctuates between 280 and 350 mm/°C.

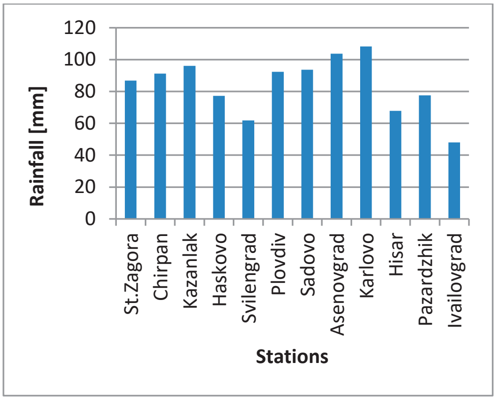

Figure 12. Average multi-annual rainfall totals [mm] in June-July

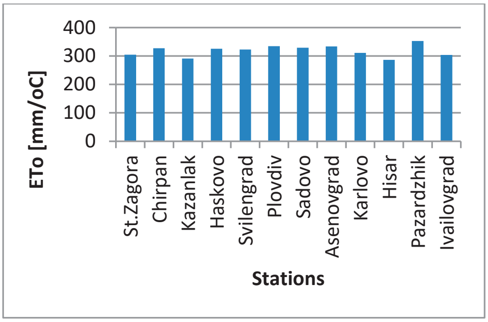

Figure 13. Average multi-annual sum of ETo [mm/°C] during the period June-July

During the period 1991-2020, the values of the Balance atmospheric moisture during the critical period June - July varied between 160 and 200 mm/°C in the northern parts of the region and 200-240 mm/°C in the rest (Figure 14).

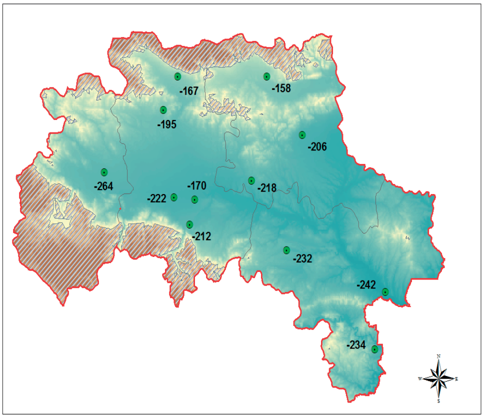

Figure 14. Sum of atmospheric moisture deficit [mm] for the months of June and July

Discussions

Central South Bulgaria region is defined as slightly favorable for growing sunflowers due to the climatic features of the region and, more specifically, the large deficit of atmospheric humidification in June and July (Soil-climatic zoning of the main field crops, 1969). The study of the agro-climatic conditions for growing sunflowers in Central South Bulgaria shows that during the last thirty-year period, there has been no statistically significant change in the amounts of rainfall and ETo during the growing season. There is no significant change in the two parameters during the critical period of inflorescence formation - flowering. The risk of drought at this time remains.

The possibilities for technological response are related to the earliest possible sowing. Because the biological minimum of the culture is 5°C, we have presented the average dates of air temperature increase above 5°C, which is in the second decade of February. Theoretically, this is the term that can be used to start sowing. In the regions with a winter maximum of rainfall, even though they have higher temperatures, this is difficult to implement due to the impossibility of preparing the areas and carrying out the sowing. When sowing at the end of February, due to the still low temperatures, germination occurs more slowly, and some of them lose their germination (Stoyanova at all., 1977). If early sowing is applied, the seeding rate should be increased. The recommended sowing dates for the region are March 25-30, but if possible, sowing can be done after the permanent retention of temperatures above 10°C. During the analysis of temperature conditions, it was found that the earliest dates with temperatures above 10°C, with a probability of once in four years, are in the southernmost regions at the end of the first decade of March, in the second decade in the rest, except for Kazanlak, where this happens on 25.03. The average dates of the increase in average daily temperatures above 10°C are in the last decade of March for most of the region and in the second decade for the southern parts. Such conditions suggest withdrawing the sowing dates as early as possible. As an alternative to increasingly severe droughts in the critical periods of crop development, the zoning of varieties with a shorter vegetation period can be recommended, which, combined with the earliest possible sowing date, will result in more guaranteed crop yields. Otherwise, the trend of droughts resulting in a large deficit of atmospheric moisture in the period of flowering-fruiting will make growing the crop riskier, and years with zero yields will be more and more common.

CONCLUSIONS

The assessment of agrometeorlogcal conditions shows that the average date of the permanent transition in 5°C and 10°C are, respectively, in the second decade of February and the last decade of March for the greater part of the region and in the second for the southern parts. The rainfall totals during the sunflower vegetation period April-July is between 33 and 53% of the amount of potential evapotranspiration, and for the critical period June-July between 16 and 35%.

There are no statistical differences in the trends of changes in rainfall amounts and potential evapotranspiration for both periods - April-July and June-July. An exception is increased rainfall in the three stations near Plovdiv and the potential evapotranspiration in Ivaylovgrad, Chirpan and Kazanlak.

Although in the northern points of the study area (Karlovo, Kazanlak), the conditions for growing sunflowers are similar to the average value for a long period. For the rest, it can be concluded that the sowing of the crop should be done as early as possible depending on the conditions in the particular year (the first or second decade of March), and it should be combined with the cultivation of varieties with -short vegetation, in which the flowering-fruiting phases are earlier when the atmospheric moisture deficit is the smallest.

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MINERAL FERTILIZATION, FOLIAR APPLICATION AND VARIETIES AS A FACTORS INFLUENCING TRITICALE (X *Triticosecale* Witt.) PRODUCTIVITY

Stefka STEFANOVA-DOBREVA, Angelina MUHOVA

Agricultural Academy, Field Crops Institute, 2 Georgi Dimitrov Blvd, 6200, Chirpan, Bulgaria

Corresponding author email: stefanovadobreva@gmail.com

Abstract

The aim of the study was to investigate the productive possibilities and the structural elements of the yield depending on the variety, the applied fertilizer rate and the import of foliar treatment for winter triticale. The study was conducted in 2019/2020-2020/2021. Colorit, Attila and Boomerang varieties were tested. Nitrogen fertilization was at rates: 0; 60 and 120 kg ha^{-1}. A foliar treatment was performed in the tillering and stem elongation phases. Mineral fertilization was a major impact on the values of all studied parameters and only for the number of grains per spike the variety has a stronger influence. The rate of 120 kg N ha^{-1} was the most productive for the observed traits. The foliar treatment was a stronger effect in the stem elongation phase, except for the plant height, where no differences were found between the two phases. Boomerang was the highest grain yield (45.0%), plant height (23.3%) and number of spikelet per spike (11.5%), Attila was the longest spike (23.4%), and Colorit was the highest number and heaviest grains per spike, 37.9% and 51.72%, respectively, compared to control.

Key words: *foliar application, mineral fertilization, triticale, varieties.*

INTRODUCTION

Although a new crop, the benefits of triticale production are enormous, and this is the reason for its adoption in more than 30 countries with an ever increasing acreage (Arough et al., 2016). Due to its biological properties and advancement in selection, production areas of triticale increase year by year, in recent years in the world triticale has been sawn on over 4 000 000 ha (Maričević et al., 2021). In Bulgaria the areas under triticale vary over the years. According to FAOSTAT, in 2000 the triticale areas were only 5 000 ha. By 2010, increased by over 50% - 11 010 ha. In 2018 they reached the highest values - 18 660 ha. According to the Ministry of Agriculture and Food in 2020 feld to 13 669 ha. Food security is a fundamental precondition for human well-being, and the agricultural and food sector is of major economic importance (Chervenkov & Slavov, 2021). However, negative changes in the environment, because of daily anthropogenic activity, are a continuous and irreversible process (Mitovska, Dimitrov & Chasovnikarova, 2021). In agriculture one of the biggest polluters is mineral fertilization. In commercial agriculture, the use of chemical fertilizers cannot be ruled out completely (Abo-Sedera et al., 2016). According Bielski et al. (2020) nitrogen fertilization was very important agronomy factor of winter triticale grain. However, investments can be reduced through deploying production technologies that are best suited to the cultivated crops and local conditions (Pawlak, 2021). One of the ways to reduce the use of mineral fertilizers is the treatment with foliar application. Foliar fertilizer application after soil fertilization is an effective method to increase to contents of trace elements in crops and crop yield, and to improve the soil environment (Niu et al., 2021). In fact, the purpose of foliar application is not to replace the mineral, but to correct specific physiological growth disorders caused by a deficiency of a particular element or group of elements. Szpunar-Krok et al. (2021) also emphasize that its most important function is in interventions to supplement nutrient deficiencies during the growing season in order to correct poor plant nutrition caused by intensive plant growth, drought, or agrotechnical errors. The authors added that the availability of macro- and micronutrients, such as Ca, S, Mg, K, N, P and Fe, is of key importance for the proper life processes of plant, which directly affects their growth and yield. The foliar application has a number of positive effects. Gamayunova et al. (2021) report that when fed with micro-fertilizers, plants become more resistant to adverse environmental conditions, pest and disease damage. The same authors point out that an important factor in increasing the yield and improving the quality of triticale grain is the use of fertilizing with micro-fertilizers. Through foliar treatment, the uptake of nutrients by plants is faster through the leaves than through the roots. Another positive quality of foliar fertilization is that the risk of leaching is reduced by applying direct treatment to the leaves.In addition, the use of foliar application as a cheap method may be recommended (Nasiru & Najafi, 2015). Timely, directed and correct impact on the processes of the root and foliar nutrition affected the course of formation and the amount of the crop (Shchuklina et al., 2021).

There is no scientific research in Bulgaria on the effect of Lactofol O foliar fertilizer on triticale. Due to the growing interest and the tendency to increase the areas of triticale, we set a goal to study the productive possibilities and the structural elements of the yield depending on the variety, the applied mineral fertilizer rate and the import of foliar treatment.

MATERIALS AND METHODS

The study was conducted in 2019/2020-2020/2021 growing seasons at the Field Crops Institute, Chirpan, Bulgaria (42°11′58″N, 25°19'27″E). A three-factor experiment was carried out using the random block method in four replications with the size of each plot of 10 m^2 after sunflower predecessor. Sowing was done with 550 germinating kernels. Three Bulgarian winter triticale varieties were tested: Colorit (standard variety); Attila and Boomerang (factor C). Fertilization with ammonium nitrate was performed in tillering phase, at rates: 0; 60 and 120 kg ha^{-1} (factor A). Fertilization with triple superphosphate was performed pre-sowing as a background in rate of 60 kg ha^{-1}.

A single foliar treatment at a dose of 6000 ml ha^{-1} was performed in the tillering (treatment 1) and stem elongation (treatment 2) phases (factor B). The treatment was performed manually, early in the morning.The foliar fertilizer (Lactofol O) contains: 21% nitrogen, including nitrate (7.5%), ammonium (4%) and amide (10%); 5% phosphorus; 10% potassium; trace elements - boron (0.02%), copper (0.014%), iron (0.25%), manganese (0.002%), molybdenum (0.002%) and zinc (0.018%). The unfertilized and untreated with mineral and foliar fertilizer variant for Colorit was used as a control.

Statistical analysis was performed by three-factors dispersion using ANOVA and the smallest mean error $p< 0.001\%$.

The soil was classified as Pellic vertisols. The soil analysis showed an organic matter content in the range of 2.4-2.9%; the soil reaction was 6.0-6.5 pH; the content of total N was 0.09%, and the presence of phosphorus and potassium - 5 ppm and 32 ppm, respectively.

Table 1 showed the meteorological conditions during the triticale vegetation for the studied period and the multi-year period. The two observed vegetation periods were a higher sum of temperatures compared to the multi-year period. With regard to precipitation, an increase in the values of the studied years was also observed compared to the 93-year period.

Table 1. Temperature sum and rainfall during the triticale vegetation for 2019/21 and multy-yearperiod

Year	Mounths								Σ
	XI	XII	I	II	III	IV	V	VI	
Temperature sum, Σ˚C									
1928-20	216.0	63.6	-3.6	58.6	191.9	355.3	507.7	624.9	2014
2019/20	336	111	45	154	257	314	516	615	2348
2020/21	197	181	99	126	162	309	524	616	2214
Rainfall, mm									
1928-20	46.9	51.7	43.7	37.7	39.2	44.2	60.5	65.5	389
2019/20	82	22	2	56	67	62	50	63	404
2020/21	7	70	109	26	39	84	35	43	413

RESULTS AND DISCUSSIONS

The analysis of the total dispersion showed that the mineral fertilization was influenced to the greatest extent by more than the parameters (Table 2).

The strongest impact was observed in lenght spike (LS) - 79.621%, followed by number of spiklet per spike (NSS) - 51.26%, grain yield (GY) - 49.01%, grain weight (GW) - 43.83%, plant height (PH) - 39.44%, respectively. Foliar application was a significant effect on most indicators, except for PH.

The variety was the most effective on number of grain per spike (NGS), and showed 57.84% of the total variation. Factor C was unproven only for LS. The conditions of the year were also of great importance for most parameters, but for GY (40.58%) were of the greatest importance. No proven interaction between the factors was observed.

From the independent action of the factors only the mineral fertilization was a proven effect for all studied parameters (Table 3). The effect of foliar application was a stronger of treatment 2 than treatment 1. No significant difference was observed only in PH. The variety was a strong impact on most traits, except LS. Proven higher values were observed only at the plant height of Attila and Boomerang, and the grain yield of Boomerang compared to the control (Colorit). Proven lower values compared to the Colorit standard were NSS, NGS and GW.

Table 2. Effect of mineral fertilization, foliar application and varieties on triticale yield and yield components (mean squares - % of total)

Source of variance	df	Grain yield, kg ha^{-1}	Plant height, cm	Lenght spike, cm	Number of spiklet per spike	Number of grain per spike	Grain weight, g
Replicate	1	40.58***	3.26*	0.47	6.98***	2.85**	19.33***
A	2	49.01***	39.44***	79.62***	51.26***	27.05***	43.83***
B	2	2.02**	0.85	4.16**	2.80*	4.40**	7.29***
C	2	2.67**	17.14***	0.52	21.71***	57.84***	13.58***
AxB	4	0.18	0.50	1.12	2.05	0.23	0.30
AxC	4	1.06	0.43	4.35*	1.58	0.30	0.38
BxC	4	0.37	0.33	0.33	0.36	0.01	0.04
AxBxC	8	0.10	0.91	1.40	1.61	0.55	0.54
Error	26	4.01	37.14	8.04	11.66	6.77	14.73
VC, %	-	4.88	5.43	3.05	2.78	7.60	9.22

A - mineral fertilization; B - foliar application; C – variety

Table 3. Independent action of mineral fertilization, foliar application and varieties on triticale average for test period

variant		Grain yield, kg ha^{-1}	Plant height, cm	Lenght spike, cm	Number of spiklet per spike	Number of grain per spike	Grain weight, g
Mineral fertilization							
No fetilizer		5139.4	110.4	11.2	30.0	55.4	2.21
N_{60}		6336.0***	120.9***	12.5***	31.9***	64.4***	2.63***
N_{120}		6870.8***	124.4***	13.1***	33.1***	71.8***	3.09***
Foliar application							
no treatment		5946.8	117.4	12.1	31.5	60.3	2.46
Treatment 1		6094.6^{NS}	118.9^{NS}	12.2^{NS}	31.4^{NS}	64.5*	2.65**
Treatment 2		6304.8**	119.4^{NS}	12.5***	32.1*	66.8***	2.82***
Variety							
Colorit		6078.3	113.3	12.3	32.2	77.3	2.92
Attila		5929.7^{NS}	119.7***	12.3^{NS}	30.5^{000}	53.9^{000}	2.46^{000}
Boomerang		6338.2*	122.7***	12.2^{NS}	32.3^{NS}	60.4^{000}	2.55^{000}
SD	*5%*	*204.5*	*2.5*	*0.3*	*0.6*	*3.3*	*0.11*
	1%	*276.4*	*3.2*	*0.4*	*0.8*	*4.5*	*0.15*
	0.1%	*368.7*	*4.2*	*0.5*	*1.1*	*6.0*	*0.20*

NS - no significant; *, **, *** significant at P = 5%, P = 1% and P = 0.1%; treatment 1 - foliar application in tillering; treatment 2 - foliar application in stem elongation

GY ranged from 4853 kg ha^{-1} (Boomerang) to 7384 kg ha^{-1} (Boomerang) and the average of all variants was 6115 kg ha^{-1} (Table 4). The varieties reacted differently to the nitrogen fertilization. This implies genotypes differ in absorption, and utilization of N depends on the environment (Belete et al., 2018). Boomerang showed that it is the most responsive to fertilization. Kucukozdemir et al. (2021) reported that, in addition to the genetic yields potential of a variety, the environmental conditions in which the plants are grown are also influence the yield. As far as foliar application treatment 2 was concerned, it was a stronger effect compared to treatment 1. Comparing the effect of mineral fertilizer rates, the N_{120} dose was a greater effect on GY than N_{60}. This result confirms the study of Wojtokowiak et al. (2015).

The authors reported the N_{120} rate as optimal. The complex action of N_{120} + treatment 2 was the strongest effect and increased GY as follows: Boomerang (45.0%), Colorit (35.6%) and Attila (33.7%). For all three varieties, plant height varied under the action of mineral fertilization. Fortunato et al. (2019) reported that with increasing fertilizer rate, the PH also increased.

The lowest value was accounted for Colorit at N_0 (103.9 cm). The tallest plants were registred for Boomerang when fertilized with N_{120}+treatment 1 (131.4 cm).

No differences in height were found with respect to foliar application introduced during the different phases.Zoz et al. (2012) confirmed that foliar fertilization of molybdenum had no proven effect on PH in wheat.

LS of the studied varieties varied from 10.3 cm (Boomerang) to 13.7 cm (Attila). The longest spike, on average for the test period, was observed for Attila, from the variant N_{120} + treatment 2, which exceeded the control by 23.4%. With a minimum difference of 0.1 cm was Boomerang. Foliar fertilizer applied in the stem elongation (treatment 2) showed a stronger effect than treatment 1. Saleen et al. (2020) also reported a positive effect of boron on LS in wheat. Again in wheat Yadav et al. (2021) reported an increase in LS under the action of $ZnSO_4$.

NSS ranged from 28.4 (Attila) to 34.1 (Boomerang) (Table 5). The highest values were obseved in fertilization with 120 kg N ha^{-1} for Boomerang, with 11.8% more than the control. The foliar application for all three varieties was a greater effect in treatment 2. In contrast to our results, Hadi et al. (2020) reported that foliar application in bread wheat with Zn and Fe had a greater effect in the tillering than in the booting phase.

Table 4. Grain yield (kg ha^{-1}), plant height (cm) and length spike (cm) average for test period

variant		GY, kg ha^{-1}	% of control	PH, cm	% of control	LS, cm	% of control
Colorit	N_0	5093	100.0	103.9	100.0	11.1	100.0
	N_0+treatment1	5217^{NS}	102.4	104.3^{NS}	100.4	11.1^{NS}	100.0
	N_0+treatment2	5508^{NS}	108.2	107.1^{NS}	103.1	11.7^{NS}	105.4
	N_{60}	6152**	120.8	115.0**	110.7	12.5***	112.6
	N_{60}+treatment1	6185**	121.4	116.2**	111.8	12.5***	112.6
	N_{60}+treatment2	6342***	124.5	117.6***	113.2	12.6***	113.5
	N_{120}	6701***	131.6	117.2***	112.8	13.0***	117.1
	N_{120}+treatment1	6605***	129.7	119.0***	114.5	13.0***	117.1
	N_{120}+treatment2	6904***	135.6	119.8***	115.3	13.3***	119.8
Attila	N_0	4859^{NS}	95.4	108.8^{NS}	104.7	11.4^{NS}	102.7
	N_0+treatment1	4903^{NS}	96.3	114.4**	110.1	11.4^{NS}	102.7
	N_0+treatment2	5169^{NS}	101.5	114.2**	109.9	11.7^{NS}	105.4
	N_{60}	6020**	118.2	121.6***	117.0	12.5***	112.6
	N_{60}+treatment1	6172**	121.2	121.1***	116.6	12.5***	112.6
	N_{60}+treatment2	6266***	123.0	121.1***	116.6	12.7***	114.4
	N_{120}	6560***	128.8	126.3***	121.6	12.6***	113.5
	N_{120}+treatment1	6611***	129.8	122.8***	118.2	12.7***	114.4
	N_{120}+treatment2	6810***	133.7	126.9***	122.1	13.7***	123.4
Boomerang	N_0	4853^{NS}	95.3	111.4*	107.2	10.3^{NS}	97.8
	N_0+treatment1	5175^{NS}	101.6	114.1**	109.8	10.9^{NS}	98.2
	N_0+treatment2	5480^{NS}	107.6	115.7**	111.4	11.1^{NS}	100.0
	N_{60}	6237***	122.5	123.9***	119.3	12.5***	112.6
	N_{60}+treatment1	6769***	132.9	126.7***	121.9	12.4**	111.7
	N_{60}+treatment2	6883***	135.2	125.0***	120.3	12.5***	112.6
	N_{120}	7049***	138.4	128.6***	123.8	13.2***	118.9
	N_{120}+treatment1	7215***	141.7	131.4***	126.5	13.3***	119.8
	N_{120}+treatment2	7384***	145.0	128.1***	123.3	13.6***	122.5
SD	*5%*	*613.4*	*12.0*	*7.4*	*7.1*	*0.8*	*7.2*
	1%	*829.2*	*16.3*	*9.7*	*9.3*	*1.0*	*9.0*
	0.1%	*1106.1*	*21.7*	*12.5*	*12.0*	*1.4*	*12.6*

NS - no significant; *, **, *** significant at P = 5%, P = 1% and P = 0.1%; treatment 1 - foliar application in tillering; treatment 2 - foliar application in stem elongation

Table 5. Number of spiklet per spike, number of grain per spike and grain weight (g) average for test period

Variant		NSS	% of control	NGS	% of control	GW, g	% of control
Colorit	N_0	30.5	100.0	65.5	100.0	2.32	100.00
	N_0+treatment1	30.3^{NS}	99.3	67.8^{NS}	103.5	2.41^{NS}	103.88
	N_0+treatment2	31.6^{NS}	103.6	69.5^{NS}	106.1	2.61^{NS}	112.50
	N_{60}	32.4*	106.2	72.0^{NS}	109.9	2.74^{NS}	118.10
	N_{60}+treatment1	32.2^{NS}	105.6	80.0**	122.1	3.00***	129.31
	N_{60}+treatment2	32.7*	107.2	81.7**	124.7	3.10***	133.62
	N_{120}	33.7**	110.5	83.6***	127.6	3.16***	136.21
	N_{120}+treatment1	33.1**	108.5	85.3***	130.2	3.43***	147.85
	N_{120}+treatment2	33.7**	110.5	90.3***	137.9	3.52***	151.72
Attila	N_0	28.4^{NS}	93.1	41.7^{NS}	63.7	1.92^{NS}	82.76
	N_0+treatment1	29.2^{NS}	95.7	47.1^{NS}	71.9	2.11^{NS}	90.95
	N_0+treatment2	29.4^{NS}	96.4	48.7^{NS}	74.4	2.23^{NS}	96.12
	N_{60}	30.5^{NS}	100.0	51.9^{NS}	79.2	2.32^{NS}	100.00
	N_{60}+treatment1	30.2^{NS}	99.0	55.1^{NS}	84.1	2.45^{NS}	105.60
	N_{60}+treatment2	31.4^{NS}	103.0	53.7^{NS}	85.2	2.53^{NS}	109.05
	N_{120}	31.5^{NS}	103.3	57.8^{NS}	88.2	2.61^{NS}	112.50
	N_{120}+treatment1	31.7^{NS}	103.9	61.6^{NS}	94.1	2.84**	122.41
	N_{120}+treatment2	32.2^{NS}	105.6	65.9^{NS}	100.6	3.15***	135.78
Boomerang	N_0	28.9^{NS}	94.8	46.8^{NS}	71.5	1.85^{NS}	79.74
	N_0+treatment1	30.6^{NS}	100.3	54.7^{NS}	83.5	2.16^{NS}	93.10
	N_0+treatment2	30.9^{NS}	101.3	56.7^{NS}	86.6	2.32^{NS}	100.00
	N_{60}	33.2**	108.9	58.4^{NS}	89.2	2.38^{NS}	102.59
	N_{60}+treatment1	32.2^{NS}	105.6	62.1^{NS}	94.8	2.54^{NS}	109.48
	N_{60}+treatment2	32.9*	107.9	62.9^{NS}	96.0	2.63^{NS}	113.36
	N_{120}	34.1***	111.8	65.0^{NS}	99.2	2.87**	123.71
	N_{120}+treatment1	33.7**	110.5	66.9^{NS}	102.1	2.96***	127.59
	N_{120}+treatment2	34.0***	111.5	70.3^{NS}	107.3	3.27***	140.95
SD	*5%*	*1.8*	*5.9*	*10.0*	*15.3*	*0.34*	*14.66*
	1%	*2.5*	*8.2*	*13.5*	*20.6*	*0.46*	*19.83*
	0,1%	*3.3*	*10.8*	*18.0*	*27.5*	*0.59*	*25.43*

NS - no significant; *, **, *** significant at P = 5%, P = 1% and P = 0.1%; treatment 1 - foliar application in tillering; treatment 2 - foliar application in stem elongation

Attila and Boomerang were significantly fewer NGS than Colorit and remained outside the statistical confidence limit. Colorit showedthe best results under the influence of N_{120} + treatment 2, exceeding the variant without fertilization by 37.9%. Tesfaye et al. (2021) confirm the results obtained in our study that the number of grains per spike was significantly influenced by the foliar treatment in bread wheat. Abdelsalam et al. (2019) observed the same trend under the influence of foliar nanofertilizers in bread wheat.

The heaviest grains were reported from Colorit (3.52 g) and the lightest from Boomerang (1.85 g). Treatmant 2 again was a stronger effect than treatment1. Parvin et al. (2020) also reported better results in the introduction of humic acid in the later stages of development than in the earlier ones. For the Boomerang variety, there was a confirmed effect only at the high rate of 120 kg N ha^{-1} alone and 120 kg N ha^{-1} with foliar application. Attila showed a significant increase only at 120 kg N ha^{-1} with foliar application. Meena et al. (2021) confirmed the effect of foliar application ($ZnSO_4$) on grain weight (GW) in bread wheat.

CONCLUSIONS

The results obtained from field experience give us reason to concluded that mineral fertilization was a major impact on the values of all studied parameters and only for the number of grains per spike the varietyhad a stronger influence.The influence of the variety was second in importance for all traits except the length spike.Foliar treatment was the least effect, but not confirmed for plant height.However, all three factors were a proven and significant impact.The rate of 120 kg N ha^{-1} was the most productive for the observed traits. Foliar treatment was a stronger effect in the stem elongation phase, except for plant height, where no differences were found between the two phases.Boomerang was the highest grain yield (45.0%), plant height (23.3%) and number of spikelet per spike (11.5%) compared tocontrol.Attila was the longest spike (23.4%).Coloritwas with the largest number and heaviest grains in spike, 37.9% and 51.72%, respectively.

Based on the results obtained from our study, we recommend that farmers fertilize with 120 kg N ha^{-1} in combination with foliar fertilizer, applied in the later stages of plant development of new, high-yielding varieties and hybrids of triticale.

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REGRESSION BETWEEN YIELD AND GROWTH INDICATORS OF COTTON VARIETIES

Antoniya STOYANOVA[1], Georgi STOYANOV[2], Svetla Kostadinova[2]

[1]Trakya University, Faculty of Agriculture, Stara Zagora, 6000, Bulgaria
[2]Agricultural University of Plovdiv, 12 Mendeleev Blvd, 4000, Bulgaria

Corresponding author email: toni_1219@abv.bg

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EVALUATION OF THE IMPACT OF SOWING SEASON AND WEATHER CONDITIONS ON MAIZE YIELD

Alina ȘIMON, Adrian CECLAN, Voichița HAȘ, Andrei VARGA, Florin RUSSU, Felicia CHEȚAN, Marius BĂRDAȘ

Agricultural Research and Development Turda, 27 Agriculturii Street, Cluj County, Romania

Corresponding author email: adi.ceclan@gmail.com

Abstract

Climate change has become the biggest global challenge to agriculture and food production. In the context of current environmental changes, the aim of this study is to identify the optimal sowing season that leads to obtaining high and constant yields. The study followed the reaction of 7 native maize hybrids to cultivation in 3 different sowing seasons, over a period of 3 years. The data obtained show us that the best yield results are obtained on mid-early hybrids (9327-9843 kg/ha). Sowing maize too early, are obtained lower yields than for maize sown at 10ºC in the soil, with a very significant difference of 1337 kg/ha. Favorable climatic conditions in 2020 and 2021 emerge from the average yields obtained in the two years, 10343 kg/ha (2020) respectively 9424 kg/ha (2021). The climatic conditions of 2022 were less favorable, summer drought having a negative effect on average maize yield, which was around 6924 kg/ha.

Key words*: sowing season, maize hybrids, yields, climate change.*

INTRODUCTION

Climate change has become the biggest global challenge, causing problems in crop development and yield, leading to threats to food security for the growing population (Bennetzen et al., 2016).

Considering the importance of agriculture in the global economy and food, the response of plants to climate change is and will be studied through different approaches.

Maize is a cereal with a high production capacity, but also with a wide spread area, being less influenced by climate change, having a high resistance to drought, heavy rains, diseases and pests, with an accessible technology, agrotechnical and harvesting works being able to be fully mechanized (Haș et al., 2018). In Romania, maize is the most extensive crop (Popescu, 2018), with a use in human and animal nutrition, that is why it is necessary to consider the achievement of a balance between the production capacity in the choice of corn hybrids and grain quality indicators (Scott et al., 2006).

It is considered a drought-resistant plant, but it responds differently to water deficit depending on the stages of development (Cakir, 2004). From sowing to harvesting, maize is subjected to many stress factors, both biotic and abiotic, these factors together make up an ecosystem (Popa et al., 2021).

For the maize crop, the following phases are distinguished as critical for water: the sprouting phase - ripening in milk-wax (7-10 days before sprouting, 10-20 days after flowering) (Botzan, 1966). Moisture stress occurring at critical growth stages (anthesis and grain filling) causes yield losses in maize (Basir et al., 2018).

The most critical period of corn for water stress is 10 to 14 days before and after flowering, with grain yield reduced two to three times more when water deficit coincides with flowering compared to other growth stages (Grant et al., 1989).

Crop production is influenced by weather effects and agrotechnical conditions, so its variability can be difficult to predict (Doré et al., 1998; Marin et al., 2012; Rusu, 2014), but by using high-yielding biological material, sowing at the right moment and using advanced agricultural practices it can be increased to a certain extent (Qureshi et al., 2007).

Each plant species has a certain temperature threshold necessary to start the activity, for maize the basic temperature is 10ºC (Rao Prasada, 2008), following that it will grow with the development of the plants and the progress in the vegetation. During the vegetation period, the temperature required by maize is between 10 and 30ºC, temperatures lower or higher than these thresholds can have negative effects on crop development.

According to the data of the National Meteorological Agency (ANM), the evolution of the heat intensity in Romania, during 1961-2010, shows an upward trend, which started in 1981, a trend that continues today (Sin & Popescu, 2015).

Seeding of the maize crop earlier than 10ºC soil temperatures may be limited by cooler soil temperatures and soil moisture conditions (Kucharik, 2008), the emergence and normal development of plants can be affected by soil and climate conditions.

Climatic changes, especially the increase in temperatures and the lack of precipitation, during the period of the formation of reproductive organs are the main cause leading to significant production losses, the adaptation of agricultural technologies being among the most accessible methods of reducing the impact of global warming on the structure of agricultural plants.

Climatic variability and unfavorable spring conditions mean that farmers to cannot sow at the optimal moment every year, there is a need to study the reaction of the maize crop to different sowing dates (season) in order to identify when maize can be sown without suffering large production losses.

Considering these aspects, the aim of the work is to study the behavior of some native corn hybrids when changing some technological elements (sowing season) as well as the interaction of these factors on the corn culture.

MATERIALS AND METHODS

The research has been developed during 2018-2021, at the Turda Agricultural Research and Development Station, on a type soil chernozem, characteristic to Transylvanian Plateau. As a chemical description, the soil has a weakly alkaline neutral pH, neutral to high humus content, well supplied in nitrogen and potassium, medium supplied in phosphorus.

In order to achieve the proposed objectives, a bifactorial experiment was organized, with the following factors: factor A - sowing season with three graduations: sowing season I - when 6ºC are recorded in the soil for three consecutive days; sowing season II - when 8ºC are recorded in the soil for three consecutive days; sowing season III - when 10ºC are recorded in the soil for three consecutive days; factor B - maize hybrids with seven graduations: Turda 248 (FAO 300); Turda 165 (FAO 270); Turda 201 (FAO 340); Turda Star (FAO 370); Turda 332 (FAO 380); Turda 344 (FAO 380); Turda 335 (FAO 380); factor C - climatic conditions in the experimental years: 2020; 2021 and 2022.

At sowing, was executed a fertilization with 150 kg/ha NPK (20:20:0), and in the phenophase of 4-6 leaves an additional fertilization with 200 kg/ha CAN (27%). The sowing density was 70,000 plants/ha. The predecessor plant was winter wheat.

The obtained results were processed statistically by the variance analysis method and establishing the smallest significant difference - DL - (5%, 1% și 0.1%) (ANOVA, 2015).

Climatic conditions are one of the most important factors that influenced the productivity of an agricultural crop, an analysis of climate factors being justified in the context in which climate change has become a global problem. The climatic data presented come from the Turda Weather Station, located on the coordinates: longitude 23º47’; latitude 46º35’; altitude 427 m.

Although there is an annual increase in the average temperature, in the spring it is observed that the temperatures are lower than normal, which leads to a slower warming of the soil and indirectly to the delay in the moment when agricultural crops can be sown under suitable conditions.

With the beginning of summer, important increases in temperature are observed, temperatures that are not beneficial to agricultural crops, especially in periods when low amounts of precipitation are recorded. The most important increases for the average temperature are found in the period June-July, with up to 3.3ºC deviation from the normal of the period (Table 1).

The rainfall regime of the three years was variable, with a deficit of precipitation in the periods when the crop has the highest consumption (July), the most important lack of precipitation being observed in 2022, while in June and July the total was 67.3 mm, less than half the normal amount for this period (Table 2).

Table 1. Average air temperature during the period 2020-2022

Experimental years	Average air temperature (ºC)						
	Month/decade	April	May	June	July	August	September
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 65 years	10.0	15.0	18.0	19.8	19.5	15.2
2021	Decade I	5.9	12.8	16.9	21.6	21.5	15.9
	Decade II	7.8	14.3	18.6	24.0	21.2	17.1
	Decade III	9.8	15.1	23.9	22.4	16.7	12.1
	Monthly average	7.8	14.1	19.8	22.7	19.7	15.0
	Average 65 years	10.0	15.0	18.0	19.8	19.5	15.2
2022	Decade I	7.4	14.7	20.4	23.0	23.0	17.0
	Decade II	7.6	16.9	20.1	21.2	22.5	14.1
	Decade III	11.4	17.1	22.9	24.9	21.4	11.9
	Monthly average	8.8	16.3	21.1	23.1	22.3	14.3
	Average 65 years	10.0	15.0	18.0	19.8	19.5	15.2

Table 2. The amount of precipitation for the period 2020-2022

Experimental years	Rainfall (mm)						
	Month/decade	April	May	June	July	August	September
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 average	17.8	44.4	166.6	86.8	58	57.4
	Average 65 years	45.6	69.4	84.6	78.0	56.1	42.4
2021	Decade I	8.1	4.4	7.2	56.7	7.4	1.5
	Decade II	18.5	32.2	25.6	63.9	7.5	11.0
	Decade III	11.8	44.2	12.2	2.5	38.0	26.6
	Monthly average	38.4	80.8	45.0	123.1	52.9	39.1
	Average 65 years	45.6	69.4	84.6	78.0	56.1	42.4
2022	Decade I	10.6	14.5	14.1	23.1	27.6	69.9
	Decade II	1.5	24.2	27.1	0.2	1.2	32.3
	Decade III	30.4	44.2	0.6	1.9	65.8	17.7
	Monthly average	42.5	82.9	41.8	25.2	94.6	119.9
	Average 65 years	45.6	69.4	84.6	78.0	56.1	42.4

RESULTS AND DISCUSSIONS

Temperature is an important environmental agent that influence the rate of growth and development of maize plants. Sowing maize at temperatures lower than 8-10ºC can lead to significant production decreases, following the results obtained in the period 2020-2022, for the maize crop sown at 6ºC in the soil, it is observed that the production decreased by 14% compared to the sowing season of 10ºC in the soil (Table 3).

Maize plants developed in conditions where the temperatures are lower immediately after emergence grow more difficult, and at very low temperatures they fail to assimilate the nutrients, having to go through a longer time to form each individual leaf.

As Parker et al (2016) also state, very early planting increases the likelihood of poor seeding conditions due to cold, wet soils, resulting in a negative impact on plant emergence, but other authors such as Coelho et al (2021) hypothesized that adjusting the sowing date is an effective strategy for mitigating the adverse effects of climatic factors by optimizing the climatic conditions during the crop growth period. Norwood (2001) stated that the yield of maize sown in early May is higher than that of maize sown in April. Some authors state that a longer growing season due to earlier sowing of the crop allows greater utilization of resources such as solar radiation, water and nutrients (Andrade et al., 2000; Tsimba et al., 2013), but other authors say that when corn is sown outside the optimal season, yield decreases are observed

(Zhou et al., 2017), as it happened in present research (-1337 kg/ha compared to the control). In addition to the genetic factor, culture technologies adapted to regional ecological conditions can make an important contribution to limiting production losses (Popa et al., 2021), but which can be variable depending on the environmental conditions during the vegetation period.

Table 3. The influence of the sowing season on maize yield

Experimental variant	Yield		± (kg/ha)	Signifiance
	kg/ha	%		
Sowing season III (control variant)	9351	100	0	c.v.
Sowing season I	8014	86	-1337	000
Sowing season II	9327	100	-24	-
	LSD (p 5%)	77		
	LSD (p 1%)	127		
	LSD (p 0.1%)	238		

The all seven analyzed hybrids behaved differently during the studied period, although they belong to close maturity groups, the highest productions are recorded in hybrids Turda 248, Turda 332, Turda 344 and Turda 335 (Table 4).
Following the results obtained in the three years of study, we can state that the mid-early hybrids capitalize very well on the environmental conditions, the yields results being higher than those of the other hybrids, by up to 20-25%, a fact observed in 2022 and by Haș Voichița et al, which states that after the year 2000, the sum of the active temperatures during the corn vegetation period increased constantly, exceeding the multiannual average, the mid-early maize hybrids fully exploiting the area's thermal resources.
Environmental conditions during the growing season did not negatively influence the number of emerged plants per unit area, even if lower temperatures were recorded in the first part of the growing season, as observed by Domokos Zsuzsa et al in 2022, early sowing did not reduce the percentage of plants that emerged compared to the optimal sowing date, but it influenced the dynamics of emergence, as that it increased the number of days with suboptimal temperatures.

Table 4. Average yield of maize hybrids sown in the three sowing seasons

Experimental variant	Yield		± (kg/ha)	Signifiance
	kg/ha	%		
Average (control variant)	8897	100	0	c.v.
Turda 248	9570	108	673	***
Turda 165	7851	88	-1046	000
Turda 201	7667	86	-1230	000
Turda Star	8384	94	-513	000
Turda 332	9327	105	430	***
Turda 344	9637	108	740	***
Turda 335	9843	111	946	***
	LSD (p 5%)	125		
	LSD (p 1%)	167		
	LSD (p 0.1%)	220		

Variation in environmental conditions during the growing season can have a major effect on yield, factors such as water stress, heat or lack of nutrients during the growing season can reduce grain yield, a fact also observed by Edmeades et al, in the year 2000, however, the response to stress depends on the intensity, duration of exposure to stress and the stage of development of the culture as observed by Wajid et al (2004), following to his executed experiences.

Important stages in a crop's development may coincide with extended mid-season drought periods, which have become a frequent occurrence in recent times, resulting in a reduction in biomass production and yield, as also observed by Raes et al. (2006).
As Hanif & Ali (2014) state, the change in the rainfall regime concurrently with the increase in temperatures during the summer is expected in the future, and by changing the sowing date, an attempt is made to avoid the interaction of the two meteorological phenomena during the vegetation period with maximum humidity requirements, with in order to reduce production losses.
The increase in temperature associated with the lack of precipitation in the summer of 2022 influenced the acceleration of the growth processes and implicitly the reduction of the period of accumulation of reserve substances in the grain, which lead to the reduction of the yield, the difference in production achieved in 2022 being -1973 kg/ ha compared to the average of the three years studied and -3419 kg/ha compared to 2020, the year when the highest average yield of the analyzed hybrids was achieved (Table 5).
Using combined empirical forecasting models, Tigchelaar et al. (2018) showed that maize production may decrease by 20-40% and 40-60% respectively if the temperature increases by 2 and 4ºC.
The climatic conditions of the first two years met the water and heat requirements for maize so that the average yield achieved by the seven hybrids was between 9424 and 10343 kg/ha, with a difference between 6 and 16 percent compared to the average of the years.

Table 5. The average production of maize obtained during the period 2020-2022

Experimental variant	**Yield**		**±**	**Signifiance**
	kg/ha	%	**(**kg/ha)	
Average years (control variant)	8897	100	0	c.v.
2020	10343	116	1446	***
2021	9424	106	527	***
2022	6924	78	-1973	000
	LSD (p 5%)	74		
	LSD (p 1%)	98		
	LSD (p 0.1%)	126		

It is known that plants need light, water and heat to grow, the requirements being different from one species to another, for maize, it needs a warm soil to germinate and grow (Bunting, 1968), corn growth at temperatures below 10ºC being slow (Lehenbauer, 1914), but from our experiment it can be seen that the seven analyzed hybrids recorded average yields between 7095 (hybrid Turda 165) and 8967 kg/ha (Turda 248) as it the maize was sown at a temperature of 6ºC in the soil, satisfactory yields obtained that the highest yield was of 8375 kg/ha (Turda 165) and 10041 kg/ha (Turda 248) in the period they were sown at 10ºC (optimal temperature in the soil) or 8ºC in the soil (Table 6).
In similar studies Baum et al. (2018) observed that in early sowing seasons there are yield increases in late maturing hybrids compared to early and mid-early hybrids. From the interaction of the two factors it is observed that the hybrids from the FAO 380 group as well as the hybrid Turda 248 records the highest yields in all three sowing seasons, with statistically significant differences compared to the control.
Following the analysis executed on the effect of the interaction between the studied hybrids and the three sowing seasons, we can state that the genotypes behave differently in similar environmental conditions, the yields differences between the experimental variants being quite high.
Maize is a thermophilic plant, with different temperature requirements depending on the vegetation stage of the plant, from 10ºC in the soil during the germination-emergence period and up to a maximum of 30ºC, the completion of the development stages in optimal conditions being limited many times of the potential of biological material to adapt to variations in temperature and precipitation.

The studied hybrids are adapted to the climatic conditions of the Transylvanian Plateau and have a good tolerance to the low temperatures in the first part of the vegetation period, their development following earlier sowing not being greatly affected by the low temperatures, only needing a larger number of days to go through the development stages. From the material selected for this experiment, the hybrids Turda 248, Turda Star and Turda 344 are noted, which register very significant and distinctly significant increases in production when they are sown at a temperature of 8ºC in the soil.

For the Turda 201 hybrid is not recommended for sowing below the temperature of 10ºC in the soil, the productions achieved with these variants having low values compared to the control, with statistically assured differences as very significant (Table 7).

Following the results obtained, Nagy (2009) finds that due to the distribution of precipitation during the vegetation period, the change of the sowing season has no influence on the performance of the hybrids, but the results obtained by Tahir et al. (2008) show us that between the maize hybrids cultivated in different sowing season there are significant yields differences.

Table 6. Influence of interaction between hybrids and sowing season on maize yield

Experimental variant	**Yield**		**±**	**Signifiance**
	kg/ha	%	(kg/ha)	
Average (control variant)	8014	100	0	c.v.
Turda 248 x Sowing season I	8967	112	953	***
Turda 165 x Sowing season I	7095	89	-919	000
Turda 201 x Sowing season I	7135	89	-878	000
Turda Star x Sowing season I	7308	91	-705	000
Turda 332 x Sowing season I	8743	109	730	***
Turda 344 x Sowing season I	8538	107	524	***
Turda 335 x Sowing season I	8309	104	296	**
Average (control variant)	9327	100	0	c.v.
Turda 248 x Sowing season II	10041	108	715	***
Turda 165 x Sowing season II	8084	87	-1243	000
Turda 201 x Sowing season II	7623	82	-1703	000
Turda Star x Sowing season II	9087	97	-240	0
Turda 332 x Sowing season II	9549	102	222	*
Turda 344 x Sowing season II	10407	112	1080	***
Turda 335 x Sowing season II	10496	113	1169	***
Average (control variant)	9351	100	0	c.v.
Turda 248 x Sowing season III	9702	104	351	***
Turda 165 x Sowing season III	8375	90	-976	000
Turda 201 x Sowing season III	8243	88	-1109	000
Turda Star x Sowing season III	8758	94	-594	000
Turda 332 x Sowing season III	9689	104	338	***
Turda 344 x Sowing season III	9967	107	616	***
Turda 335 x Sowing season III	10725	115	1374	***
	LSD (p 5%)	196		
	LSD (p 1%)	260		
	LSD (p 0.1%)	335		

Table 7. Influence of the interaction between sowing season and hybrids on maize yield

Experimental variant	Yield		±	Signifiance
	kg/ha	%	(kg/ha)	
Sowing season III x Turda 248 (control variant)	9702	100	0	c.v.
Sowing season I x Turda 248	8967	92	-735	000
Sowing season II x Turda 248	10041	104	339	**
Sowing season III x Turda 165 (control variant)	8375	100	0	c.v.
Sowing season I x Turda 165	7095	85	-1280	000
Sowing season II x Turda 165	8084	97	-291	0
Sowing season III x Turda 201 (control variant)	8243	100	0	c.v.
Sowing season I x Turda 201	7135	87	-1107	000
Sowing season II x Turda 201	7623	93	-619	000
Sowing season III x Turda Star (control variant)	8758	100	0	c.v.
Sowing season I x Turda Star	7308	84	-1449	000
Sowing season II x Turda Star	9087	104	329	**
Sowing season III x Turda 332 (control variant)	9689	100	0	c.v.
Sowing season I x Turda 332	8743	90	-946	000
Sowing season II x Turda 332	9549	99	-140	-
Sowing season III x Turda 344 (control variant)	9967	100	0	c.v.
Sowing season I x Turda 344	8538	86	-1429	000
Sowing season II x Turda 344	10407	104	440	***
Sowing season III x Turda 335 (control variant)	10725	100	0	c.v.
Sowing season I x Turda 335	8309	78	-2415	000
Sowing season II x Turda 335	10496	98	-229	0
LSD (p 5%)	213			
LSD (p 1%)	292			
LSD (p 0.1%)	404			

CONCLUSIONS

The obtained results reveal the fact that early sowing in the climatic conditions of the Transylvanian Plateau, even if it manages to avoid the negative impact of the stress factors during the vegetation period, does not manage to bring a benefit of yields, the average yields recorded when crop is sow at 6ºC in the soil are only being significantly lower than those obtained in the other two sowing season.

Sowing maize at a soil temperature of 8ºC can be an alternative option for sowing maize, some of the studied hybrids registering slightly higher yields compared to those obtained in the variant when sowing was executed at 10ºC temperature in soil

The modification of certain technological steps in order to achieve satisfactory yields can be a method of adaptation to climate change if the requirements of the plants for biological factors are respected.

Since weather conditions are not easy to predict and their effect on agriculture can be devastating, it is necessary to draw up sustainable management strategies in order to adapt to future climate changes.

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USE OF ACETAMIPRID IN THE MANAGEMENT OF *Athalia rosae* POPULATION FROM OILSEED RAPE AGROECOSYSTEM

Ramona ȘTEF[1], Ioana GROZEA[1], Otilia COTUNA[1], Veronica SĂRĂȚEANU[1], Maria IAMANDEI[2], Anișoara DUMA COPCEA[1], Lenuța Iuliana EPURE[3], Dan MANEA[1], Alin CARABET[1]

[1]University of Life Sciences "King Mihai I" from Timisoara, 119 Calea Aradului Street, Timisoara, Romania
[2]Research - Development Institute for Plant Protection Bucharest, Romania
[3]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd., District 1, Bucharest, Romania

Corresponding author email: maria_iamandei@yahoo.com

Abstract

Athalia rosae larvae attack can lead to complete defoliation of the plant leaving untouched the main veins. Knowing these aspects, in the western part of Romania, research was carried out aiming to reduce the population of Athalia rosae using acetamiprid applied in four doses (0.04 kg/ha; 0.06 kg/ha; 0.08 kg/ha ; 0.1 kg/ha). The effectiveness of the treatments in respect of larval population reduction was determined at 3, 6 and 9 days after application. At the time of treatments spraying, the population level of Athalia rosea showed close and statistically undifferentiated values, between 0.23 and 0.4 larvae/plant. It was observed that, both, the period and the treatment, had a real influence on the number of larvae during the study. Six days after the treatment, the number of larvae was significantly reduced, followed by a increases in the next period. During the study, the treatment applied at 0.08 kg/ha exerted the highest efficiency in terms of Athalia rosea larvae control, registering values of 95.70 and 90.18% after six and nine days after application.

Key words*: Athalia rosae, synthetic pyrethroids, acetamiprid, oil seed rape, management.*

INTRODUCTION

Oilseed rape (OSR - *Brassica napus*), is the main oilseed crop in the European Union, along with sunflower and soybean (FAO, 2023). The large OSR growing countries in Europe, which provide approximately 80% of the production, are: France, Germany, Poland, the United Kingdom, Romania, the Czech Republic (AHDB, 2018).

In Romania, the popularity of rapeseed culture is continuously increasing. According to the National Institute of Statistics, the cultivated area of rapeseed increased by 21 thousand hectares in 2022 compared to 2021. The increase being from 446 thousand hectares in 2021 to 467 thousand hectares, representing an increase of 4.70% (https://insse.ro/cms/sites/default/files/com_presa/com_pdf/prod_veg_r22.pdf). In Timiș County, in 2023, the cultivated surface increased by 14,246.17 ha, reaching from 51,306.05 hectares in 2022 to 65,552.22 hectares in 2023, an increase of 27.76%.

The success of establishing agricultural crops as well as guaranteeing production is conditioned by climatic conditions and compliance with the technological links of plant protection against harmful organisms (weeds, pathogens, insects) (Chiriță et al., 2004; Ștef et al., 2013; Paraschivu et al., 2021; Velea et al., 2021; Zală et al., 2023, Ștef et al. 2022). Scientifical paperwork shows that with the increase in the areas cultivated with rapeseed, the attack of harmful species has also increased, which is constantly on the rise. In Romania, in the last twenty years, studies on the harmful entomofauna of rapeseed culture have been carried out by: Trotuș et al., 2001; Trotuș, 2007; Trotuș et al., 2008; Trotuș et al., 2009; Tălmaciu et al., 2010; Buburuz et al., 2012; Rîşnoveanu and Burtea, 2012; Rîşnoveanu et al., 2012; Popovici and Tălmaciu, 2013; Răileanu and Tălmaciu, 2014; Raicu and Mitrea, 2019; Trotuș et al., 2019; Apostol et al., 2020; Trotuș et al., 2020; Vîrteiu et al., 2022; Georgescu et al., 2023.

These harmful organisms can cause losses of 35% (Trotuș et al., 2008), sometimes even higher (over 60%). The pest complex includes approximately 48 species of the class Insecta Systematically, these insects belong to the following orders: Coleoptera 25 species; Lepidoptera 9 species; Diptera 6 species; Heteroptera 4 species; Hymenoptera 1 species; Homoptera 3 species (Răileanu and Tălmaciu, 2014; Apostol et al., 2020). According to the studies carried out by Ursache et al. (2017) Coleoptera species represent 77%, and those belonging to the Lepidoptera order 6%. This situation is similar in other European countries.

In autumn, the main pests that attack OSR crops, in Romania, are *Phyllotreta* spp. *Psylliodes chrysocephala*, *Athalia rosae* and *Pieris brassicae* (Trotuș et al., 2009; Rîșnoveanu et al., 2012; Buburuz, 2012; Trotuș et al., 2019).

Athalia rosae (Hymenoptera: Tenthredinidae) is one of the species that can cause significant damage to canola crops, especially in warm and dry autumns. The damage stage is the larval stage, when an individual consumes, in 24 hours, twice its body weight (Raicu and Mitrea, 2019), a fact that can lead to a very accelerated rate of defoliation. Having two generations per year, the attack takes place in spring and autumn as well. The spring attack is not so dangerous for the rape crop. The most dangerous is when the rape crop is in the first stages of development and the climatic conditions are favourable. The lack of monitoring of the OSR crop, in the first part of the vegetation period, as well as the failure to apply insecticides can compromise/lose the entire crop. To control the main pests in the OSR crops, Romanian farmers relied on seed treatment with neonicotinoids or foliar treatments with neonicotinoids and insecticides pyrethroids (Wang, 2021; Trotuș, 2001; Georgescu, 2015). Seed treatment with neonicotinoid insecticides was the most effective method of protecting rapeseed crops in Romania against pests that attack this crop in the early stages of vegetation in autumn (Trotuș, 2009; Trotuș, 2019; Trașcă, 2019). After the ban of neonicotinoids in the EU in 2018 (Commission implementing regulation, 2018), the only active ingredient available for the treatment of rapeseed in Romania remains cyantraniliprole. This is an insecticide of the ryanoid class, authorized in November 2017, used for the treatment of rapeseed in autumn for the control of the species: *Phyllotreta* spp., *Psylliodes chrysocephala*, *Athalia rosae* and *Delia radicum* (National Commission for the Registration of Plant Protection Products, 2023). At the same time, only a few active ingredients from the pyrethroid class remained available for foliar treatments in OSR crops to control the main pests in autumn or spring. These facts can affect the costs of controlling these pests (Kathage, 2018). Without effective control methods, the canola pest population may increase in the future (Ortega-Ramos, 2022). In the coming years, most insecticides are likely to be banned due to the European Union's Green Deal policies to halve the use of chemical pesticides by 2030 (Prandecki, 2021; Tataridas, 2022).

The chemical method is the most used to control this pest, applying mainly insecticides from the group of synthetic pyrethroids and neonicotinoids (Brandes et al., 2018). Acetamiprid is an odorless neonicotinoid insecticide with a chloropyridinyl group that acts on acetylcholine receptors (nACh), which are used to combat pests present on fruit trees, vegetables, ornamental plants (Shi et al., 2011; Wang et al., 2021**)**. It is an organic compound with a molar mass of 222.67 g/mol and the chemical formula $C10H11ClN4$. This neonicotinoid insecticide is usually a xenobiotic and can have harmful effects on non-target insect predators and vertebrates (Fogel et al., 2013; Padmavathi et al., 2021; Su et al., 2022). Consequently, acetamiprid, effective on several insect pests, may have harmful side effects and should be used sustainably at low concentration. Therefore, the present study was conducted to control the population of *Athalia rosae* with the most effective dose of acetamiprid by applying a low concentration.

MATERIALS AND METHODS

Site location. The research was carried out in the western part of Romania, near Șag (45.640843º 21.174 8 21º), Timiș county, in year 2020/2021 (Figure 1).

Figure 1. Trial location
(image retrieved https://www.google.com/maps/place/%C8%98ag and processed by Ștef)

The site where the research was carried out is characterized by a moderate continental climate, with slight Mediterranean influences. The average annual temperature is 10.9°C, and the annual precipitation rate is 623 mm (Ștef et al., 2020; Ștef et al., 2022). The water need of the crops can be supplied by Timis river flowing nearby trough irrigation systems (Lazu et al., 2019). The soil is favourable for the cultivation of agricultural plants, mainly cereals, technical and fodder plants.

Experimental design

The study regarding the control of the *Athalia rosae* species, from the rapeseed agroecosystem, consisted by five treatments, untreated control included and four were treated with acetamiprid applied in different doses (0.04 kg/ha; 0.06 kg/ha; 0.08 kg /ha; 0.1 kg/ha). The experiment was arranged in a randomized block design (RBD), with 4 replicates for each treatment (Figure 2). Plot net size was 30 m^2.

Figure 2. The experimental plots (photo Carabet Alin, 2020)

The OSR hybrid used in the experiment was Umberto KWS, drilled on 18.08.2020.

The application of acetamiprid-based treatments was performed when the rapeseed plants were in the BBCH 12-14 growth stage (19.09.2020) (Figure 3).

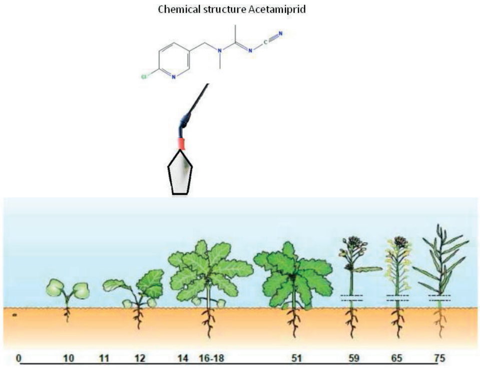

Figure 3. BBCH stage of application of acetamiprid treatments (image taken https:/pubchem.ncbi.nlm.nih.gov/compound/213021#section=2D-Structure/ http://www.g c ic -global.com/oilseed-rape/ and processed by Ștef)

Chemical treatments were applied when the turnip sawfly exceeded the economic damage threshold (P.E.D. - 2 larvae/plant) (Figure 4).

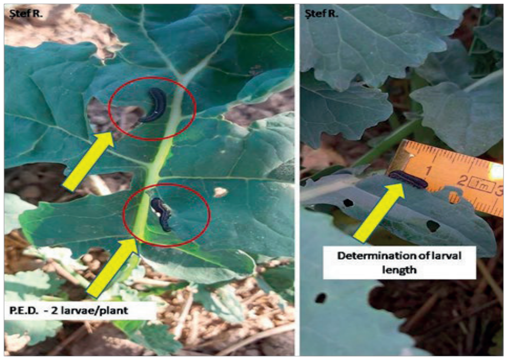

Figure 4. Assessment of *Athalia rosae* population of before the application of treatments (photo by Stef, 2020)

Damage and efficacy assessments

On the day of applying the acetamiprid-based treatments, 25 plants/plot were marked (Figure 5), after which the number of attacked plants, the number and size of *Athalia rosae* larvae and the intensity of the attack were determined (Figure 6).

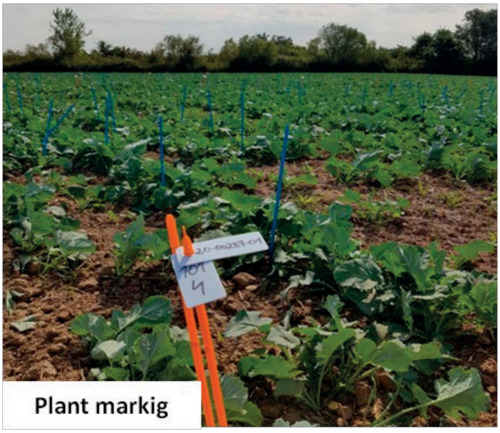

Figure 5. Marking of rapeseed plants that were observed throughout the experiment (Photo Carabet, 2020)

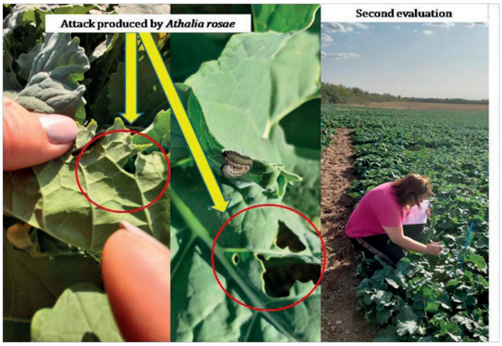

Figure 6. Assessing the attack frequency and population of *Athalia rosae* (Photo Ștef R., 2020)

Following applications of the treatments, the efficacy of the insecticides against *Athalia rosae* larvae was determined. Three assessments were performed: 3 days after application (22.09.2020), 6 days (25.09.2020) and 9 days after treatments (28.09.2020).
The intensity of the attack was determined visually for the whole plant affected, using the EPPO rating scale.

Statistical analysis
The data were subjected to statistical analysis using ANOVA, and the differences between treatments were tested by least significant difference (LSD) test at 5% significance level (Ciulca, 2006).
The efficacy of treatments was calculated by Henderson-Tilton's formula (Henderson and Tilton 1955) using the following equation: E (%) = [1-(Ta/Ca) × (Cb/Tb)] ×100, where: Ta - number of larva after treatment; Tb - number of larva before treatment; Ca - number of larva in control plots after treatment; Cb - number of larva in control plots before treatment.
Population Density Change (PDC %) = [(Ni−N0)/N0] × 100, where: N0 - mean number of larva before treatment; Ni - mean number of larva after treatment.

RESULTS AND DISCUSSIONS

Taking into account the results of the analysis of variance (Table 1), can be observed that both the period and the treatment had a real, distinctly significant influence on the number of larvae during the study, in conditions of homogeneity between the repetitions. The dose of acetamiprid showed the highest contribution to the variability of the number of *Athalia rosea* larvae, significantly superior to the effect of the period. Likewise, the interaction between the two factors showed significant influences on the variation in the number of larvae, but considerably less than the separate effects of the factors. Regarding the affected surface/plant (disease on surface), significantly higher variations are found between assessments, compared to the variations between the different treatments. Overall, the variability of the affected surface/plant was influenced to a lesser extent by the studied factors, compared to the number of *Athalia rosea* larvae.

Table 1. ANOVA for larva number ATALCO and disease on surface of *Brassica napus* plants

Source of variation	DF	Larva number		Disease on surface (%)	
		MS	F value	MS	F value
Replication	3	0.02	0.98	1.97	1.40
Period	3	0.94	59.00**	42.33	30.25**
Treatment	4	1.36	84.81**	15.39	11.00**
Period x Treatment	12	0.31	19.45**	3.25	2.32*
Residual	57	0.02		1.40	

*Significant at $p < 0.05$; ** Significant at $p < 0.01$.

Regarding the effect of the treatment, (Table 2) mean values of the number of *Athalia rosea* larvae/plant were found with the limits from 0.27 in the case of the dose of 0.08 kg/ha of acetamiprid to 0.97 in the case of the control. In general, under the effect of the different treatments, a significant reduction in the number of larvae/plant was observed compared to the control variant. Under the effect of the dose of

0.4 kg/ha, a significant reduction in the number of larvae is observed compared to the doses of 0.08-0.1 kg/ha.

Table 2. Variation for number of *Athalia rosea* larva per *Brassica napus* plant under the effect of different treatment with acetamiprid during different period of evaluation

Treatment Acetamiprid (kg/ha)	Days after treatments 0	3	6	9	Treatment mean
Control	0.32±0.03 a	1.00±0.11 a	0.93±0.04 a	1.63±0.14 a	0.97±0,13 A
0.04	0.28±0.04 a	0.89±0.09 ab	0.10±0.01 b	0.38±0.08 b	0.41±0,05 B
0.06	0.23±0.04 a	0.76±0.21 b	0.08±0.03 b	0.28±0.03 bc	0.34±0,07BC
0.08	0.40±0.07 a	0.43±0.07 c	0.05±0.02 b	0.20±0.02 c	0.27±0,08 C
0.1	0.30±0.05 a	0.51±0.03 c	0.11±0.02 b	0.28±0.05 bc	0.30±0,04 C
Period mean	0.31±0,02 Z	0.72±0,06 X	0.25±0,08 Z	0.55±0,13 Y	0.46±0,05

Period - $LSD_{5\%}$=0.09; Treatment - $LSD_{5\%}$=0.08; Treatment x Period - $LSD_{5\%}$=0.18
Values represents mean ± SE. Values with different letters (a, b) in the column indicate a significant variation at $p<0.05$. For comparisons of period's means (X, Y, Z) and treatment's means (A, B, C) capital letters were used

Regarding the assessmnet period, at the level of the whole experiment it is found that three days after the treatment the number of larvae was significantly higher than in the other periods. Also, six days after the treatment, the number of larvae is significantly reduced, to then increase in the last period.

At the time of application, the level of infestation with *Athalia rosea* presented close and statistically undifferentiated values between 0.23 and 0.4 larvae/plane.

Three days after the treatment DAT, there are significant variations in the number of larvae/plant, from 1 in the case of the control variant to 0.43 for the dose of 0.08 kg/ha. So that in this period under the effect of the doses of 0.08-1 kg/ha, the number of larvae was significantly reduced compared to both the control version and the doses of 0.04-0.06 kg/ha.

At 6 DAT, the doses of acetamiprid showed the highest effect on the larvae of *Athalia rosea*, against the background of a significant reduction compared to the control variant and compared to the evaluations in the other periods. Between the doses applied, there were no significant variations in terms of their effect, against the background of an amplitude of the number of larvae from 0.05 to 0.11.

At 9 DAT, the number of larvae/plant showed values between 0.2 for the dose of 0.08 kg/ha and 1.63 for the control variant. During this period, the the dose of 0.08 kg/ha determined a significant reduction in the number of larvae compared to the dose of 0.04 kg/ha, otherwise there were no significant variations between the other doses. All four studied doses generated a significant decrease in the number of larvae compared to the control variant.

Regarding the untreated check in Figure 7A, it is observed that during the study the number of larvae/plant varied from 0.32 to 1.63, against the background of a significant increase 3 days after treatment, an insignificant variation between 3 and 6 days and subsequently a significant increase in the last period. Also, the degree of infestation in the plot is more homogeneous at the beginning of the evaluation and 6 DAT.

Under the effect of the dose of 0.04 kg/ha (Figure 7B), the number of larvae/plant presented an amplitude of 0.1 after 6 DAT and respectively 0.89 after 3 DAT. As such 3 DAT a significant increase in the number of larvae is observed, followed by a consistent reduction at 6 DAT and then a new increase in the last period.

The research carried out by Trotuș et al. (2020) claims that by applying treatments with Imidacloprid, Clothianidin and Thiamethoxam they reduced the density of the *Athalia rosae* species very significantly compared to the untreated control.

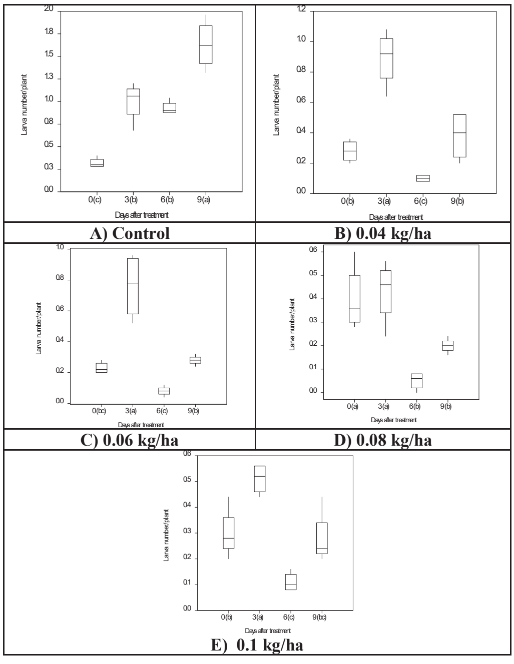

Figure 7. Boxplots of *Athalia rosea* larva number/plant for different treatments (A-E) with acetamiprid
Different letters (a, b, c) indicate significant difference at $p < 0.05$

For the plots treated with 0.04 kg/ha, the *Athalia rosea* attack was more uniform after six days and considerably more heterogeneous at the end of the evaluation.

Related to the application of the treatment with 0.06 kg/ha (Figure 7C), an irregular variation in the number of larvae/plant was observed, from 0.28 at the beginning of the evaluation, followed by a significant increase up to 0.89 after three days , then a strong decrease to 0.1 after six days and finally a new significant increase to 0.38. The variability of the attack on the plant was higher at three days and more homogeneous in the last two periods.

Regarding the treatment with 0.08 kg/ha in Figure 7D, it can be observed that, during the study, the number of larvae/plant remained relatively constant (0.4-0.43) at the first two evaluations, against the background of a significant reduction at 6 days after treatment, and subsequently a significant increase in the last period. Also, the degree of infestation in the plot was more homogeneous at 6 and 9 DAT.

Under the effect of the dose of 0.1 kg/ha (Figure 7E), the number of larvae/plant showed an amplitude of 0.11 after 6 days and up to 0.51 after three days of application. As such, 3 DAT a significant increase in the number of larvae is observed, followed by a high reduction after six days and then a slight increase in the last period. For the plots treated with 0.04 kg/ha, the *Athalia rosea* attack was more uniform after six days and considerably more heterogeneous at the beginning and end of the evaluation.

Table 3. Variation for disease on surface of *Brassica napus* plant under the effect of different treatment with acetamiprid during different period of evaluation

Treatment Acetamiprid (kg/ha)	Days after treatments				Treatment mean
	0	***3***	***6***	***9***	
Control	3.34±0.43 a	5.80±0.44 a	6.11±0.99 a	9.20±1.02 a	6.11±0.64 A
0.04	2.48±0.35 a	5.59±0.36 a	5.26±0.54 a	5.75±0.39 b	3.47±0.42 C
0.06	2.48±1.17 a	4.57±0.99 ab	4.61±1.33 ab	5.55±1.22 b	4.30±0.39 BC
0.08	2.09±0.19 a	3.27±0.77 b	3.41±0.48 b	5.11±1.02 b	4.77±0.39 B
0.1	2.08±0.39 a	4.33±0.63 ab	4.47±0.48 ab	5.87±0.20 b	4.19±0.40 BC
Period mean	2.49±0.19 Z	4.71±0.30Y	4.77±0.33 Y	6.30±0.44 X	4.57±0.22

Treatment x Period - $LSD_{5\%}$=1.68
Values represents mean ± SE. Values with different letters (a, b) in the column indicate a significant variation at $p<0.05$. For comparisons of period's means (X, Y, Z) and treatment's means (A, B, C) capital letters were used

The mean values of the affected surface/plant under the effect of different doses of acetamiprid showed an amplitude of 2.64%, with the limits from 3.47% in the case of applying the dose of 0.04 kg/ha to 6.11% in untreated, against the background of a medium variability between treatments (Table 3). Compared to the untreated plots, it is found that the application of different doses allowed a significant reduction of the surface affected by the attack. In general, changing the doses of acetampiride had small and insignificant effects on this attack indicator. However, it is found that under the effect of the treatment with 0.08 kg/ha, a significant decrease of the affected surface was manifested compared to the treatment based on 0.04 kg/h.

Very good results in terms of reduction of leaf area attacked by *Athalia rosae* were obtained by applying the Lumiposa 625 FS treatment - 11.4 l/t (3.7%) (Trotuș et al., 2020; Raicu and Mitrea, 2019).

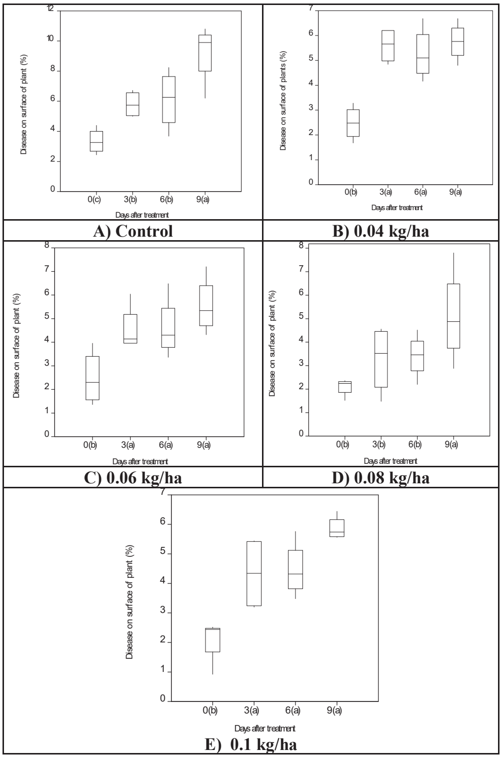

Figure 8. Boxplots for disease on surface of plant for different treatments with acetamiprid
Different letters (a, b, c) indicate significant difference at $p < 0.05$

Overall, at the level of the entire experiment, a significant increase in the affected area/plant is found after three days of monitoring, these damages are kept at a constant level up to 6 days, so that the affected area will be significantly higher at the end of the

evaluation. At the beginning of the evaluation, when the treatments were applied, the affected surface/plant showed significantly equal values between 2.08 and 3.34%.
After 3 DAT, the doses of acetamiprid showed a faded effect on the affected areas/plant, on the background of a significant reduction only in the case of the treatment with 0.08 kg/ha, which was significantly higher than the treatment with the dose of 0.04 kg/ha. Between the other doses, there were no significant variations in terms of their effect.
Six days after the treatment, the affected surface/plant showed values between 3.41% for the dose of 0.08 kg/ha and 6.11% for the control variant. In this period, the application of the dose of 0.08 kg/ha was the most effective, causing a significant reduction of the affected area/plant compared to the dose of 0.04 kg/ha, otherwise no significant variations were manifested between the other doses.
At the end of the evaluation, 9 DAT, significant reductions of the affected surface/plant are found, compared to the untreated check, against the background of small and insignificant variations between the different doses applied.
In the case of the untreated variant (Figure 8A), the affected surface/plant showed an amplitude from 3.34% at the first evaluation to 9.2% nine days after application. As such, 3 DAT, a significant increase in the affected surface is observed, relatively constant even 6 DAT, in order to record a new increase in the last period.
For the plots of the untreated variant, the affected/plant surface was more uniform in the first two evaluations and considerably more heterogeneous at the end of the evaluation.
Regarding the treatment with 0.04 kg/ha from Figure 8B, it can be observed that during the study the affected surface/plant registered a significant increase after three days from the treatment, to subsequently remain relatively constant (0.4-0.43) until the end 5.26-5.75%. Also, the degree of infestation in the plot was more homogeneous at the first evaluation and more heterogeneous after six days from the treatment.
Under the effect of dose 0.06 kg/ha (Figure 8C), a progressive variation of the affected area/plant is found, from 2.48% at the beginning of the evaluation, followed by a significant increase up to 4.57% after three days, then a constant evolution up to 4.61% after six days and finally a new increase up to 5.55%. The variability of the attack on the plant was higher at the first evaluation and more homogeneous after three days of treatment.
For the treatment with 0.08 kg/ha in Figure 8D, can be observed that the affected area/plant showed small and insignificant variations of 2.09-3.41% in the first three evaluations and subsequently a significant increase in the last period. Also, the degree of infestation in the plot was more homogeneous at the beginning of the evaluation and more heterogeneous nine days after the treatment.
When applied at 0.1 kg/ha (Figure 8E), the affected surface/plant presented an amplitude of 0.92% at the first evaluation and respectively 5.87% after nine days of application. As such, in the evaluations after the application of the treatment, a significant increase of the affected surface compared to the initial attack was found, against the background of small variations from one period to another.
For the plots treated with 0.1 kg/ha, the *Athalia rosea* attack was more uniform at the end of the evaluation and considerably more heterogeneous after three days.

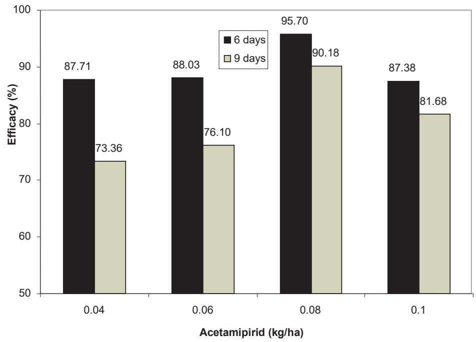

Figure 9. Efficacy of acetamipirid treatments against *Athalia rosea* larva on *Brassica napus*

Considering the data in Figure 9, can be observed that the treatment with 0.04 kg/ha exerted a superior efficiency of 87.71% at 6 DAT and an efficiency of 73.36% after nine days. In the case of the treatment with 0.06 kg/ha, a similar trend is manifested, based on an efficiency of 88.03% after six days and respectively 76.10% after nine days of application. During the study, the treatment with the dose of 0.08 kg/ha showed the highest

efficiency in control of *Athalia rosea* larvae, exerting values of 95.70 and 90.18% 6 and 9 DAT. Against the background of an efficiency of 81.68-87.38%, the treatment with 0.1 kg/ha proved superior to the doses of 0.04-0.06 kg/ha. Based on the results of the variance analysis, in table 4, can be observed that in homogeneous experimental conditions at the replicates level, the treatment with acetamipyrid significantly influenced the population density of *Athalia rosea* only three days after the treatment.
The insecticides applied, by Buburuz et al. (2012), against the species *Athalia rosae* had a good efficacy, between 90.2% (Warrat 200 SC - 0.1 l/ha) and 94.3% (Proteus 110 OD - 0.4 l/ha), at a frequency of attacked plants in the untreated control of 53.5%.

Table 4. ANOVA for change of *Athalia rosea* population density after treatment

Source of variation	DF	3 days after		6 days after		9 days after	
		MS	F value	MS	F value	MS	F value
Replication	3	6732	0.91^{NS}	506	2.23^{NS}	1402	0.72^{NS}
Treatment	3	49298	6.65*	555	2.45^{NS}	4604	2.37^{NS}
Residual	9	7418		227		1942	

*Significant at $p < 0.05$; NS - No significant at $p < 0.05$.

In terms of *Athalia rosea* population dynamics from Table 5, an increase in the density of larvae/plant can be observed three days after the application of the treatments, with variations from 18.04 to 245.69%. Thus, in the plots where doses of 0.08-1 kg/ha were applied, a significantly lower increase in population density is observed compared to doses of 0.04-0.06 kg/ha, or compared to the control variant. Six days after the treatment, all the doses applied caused a significant reduction in the population density of *Athalia rosea*, compared to the initial value, from -57.60% for the dose of 0.1 kg/ha to - 84.73% for the dose of 0.08 kg/ha. After nine days, under the effect of the treatment with 0.08 kg/ha, the population density of *Athalia rosea* was reduced by 44.61% compared to when the treatment was applied. In the case of the other treatments, at the end of the evaluation the populations of *Athalia rosea* showed a higher density with values from 7.92% for the dose of 0.1 kg/ha, up to 30.90% in the case of the dose of 0.04 kg/ha. As such, the treatment with acetamipyrid showed the highest efficacy against the larvae of *Athalia rosea*, six days after the treatment. Later, the pest populations showed a higher density.

Table 5. Change of *Athalia rosea* population density after treatments

Acetamiprid (kg/ha)	Days after treatment		
	3	***6***	***9***
Control	227.32	194.46	421.34
0.04	245.69 a	-63.96 a	30.90 a
0.06	226.55 a	-64.52 a	22.74 a
0.08	18.04 b	-84.73 a	-44.61 a
0.1	81.10 b	-57.60 a	7.92 a
$LSD_{5\%}$	*137.77*	*24.08*	*70.49*

Different letters (a, b) indicate significant difference at $p < 0.05$

Based on the data shown in Figure 10, it can be seen that the length of the *Athalia rosea* larvae at the time of application of the treatments varied from 1.76 in the case of plots where the dose of 0.06 kg/ha was applied, to 2.61 in the case of related plots treatment with 0.08 kg/ha. It is also observed that the intra-population variability for this character recorded close values between the experimental plots. As such, considering the insignificant differences between the sizes of the larvae in the trial plots, it can be concluded that the efficiency of the application of the different treatments was not influenced by the developmental stage of the *Athalia rosea* larvae.

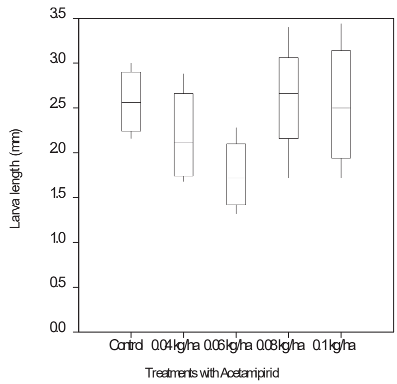

Figure 10. Boxplots of *Athalia rosea* larva length for plots of different treatments with acetamiprid

CONCLUSIONS

The four doses (0.04 kg/ha; 0.06kg/ha; 0.08 kg/ha; 0.1 kg/ha) significantly reduced the population of *Athalia rosae* compared to the untreated control.

The best efficacies in controlling the population of *Athalia rosae* were recorded in plots treated with doses of 0.08 kg/ha and 0.1 kg/ha.

The maximum period of acetamiprid effectiveness in reducing the population of *Athalia rosae* was six days after application. The size of the larvae did not influence the efficacy of the treatments.

The active ingredient acetamiprid protects the OSR crop very well for six days, after this time interval, its protection decreases.

The attacked leaf surface showed significantly lower values at the doses: 0.06 kg/ha; 0.08 kg/ha and 0.1 kg/ha.

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WINTER BARLEY GRAINS QUALITY VARIATION UNDER WATER-LIMITING CONDITIONS

Liliana VASILESCU[1], Eugen PETCU[1], Lidia CANĂ[1], Elena PETCU[1], Cătălin LAZĂR[1], Alexandrina SÎRBU[2], Silviu VASILESCU[1], Lenuța Iuliana EPURE[3], Maria TOADER[3]

[1]National Agricultural Research and Development Institute Fundulea, 1 N. Titulescu Street, Fundulea, Romania
[2]"Constantin Brâncoveanu" University of Pitesti, FMMAE Ramnicu Valcea, 39 Nicolae Bălcescu Blvd, Ramnicu Valcea, Romania
[3]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: svvasilescu@gmail.com

Abstract

The variation of some grain quality parameters has been caused by the use of a desiccant within a set of 16 winter barley genotypes (varieties and advanced lines), which led to the characterization of the indices, namely the one thousand kernel weight (TKW), protein content (P), starch content (S) and seed size (S I+II). These induced drought conditions have provided results regarding the possibility of growing certain genotypes under restrictive water conditions (field conditions) that can register both high yield and appropriate grain quality parameters. The performed analysis of the results revealed significant differences between genotypes, in terms of the influence of treatment applied and also the interaction between genotype and treatment. Results have been obtained regarding the identification of valuable genetic resources for the translocation of assimilates in water-limiting conditions but also the characterization of genotypes concerning the high stability of quality indices. These genotypes have a high content of carbohydrates in the stem and leaves at the beginning of the grain-filling period but also a high rate of translocation to the grains.

Key words: *winter barley varieties, quality indices, drought, desiccation.*

INTRODUCTION

The yield level of cereals decreases significantly during the period with low rainfall as a result of the stress caused by drought and due to the varieties being less adapted to this phenomenon, therefore the availability of water is one of the factors that significantly limit productivity (Robinson et al., 2016). Also, the thermal stress manifested after anthesis leads to some changes regarding the grain weight and size and accumulation of seed compounds which can negatively influence the grain quality (Martínez-Subirà et al., 2021).

Among the most effective methods to simulate drought stress in different species, in the postanthesis phase, chemical desiccation is recommended because it inhibits photosynthesis and thus manifests the ability to fill the grains with reserves from the stem and leaves (Blum, 1983).

When plants are treated with different desiccants such as magnesium chloride $Mg(ClO_4)_2$, sodium chloride ($NaClO_3$), potassium iodide (KI), potassium chlorate ($KClO_3$), the varieties that translocate a greater amount of carbohydrates reserve to grains are able to maintain a stable grain weight under drought conditions.

Dogan et al., in 2012, studied the effect of post-anthesis stress in the mobilization of reserves from the stem to grains in triticale and showed that some lines were more drought resistant than the other genotypes in respect of the rate of grain reduction (using potassium chlorate as desiccant agent).

During the 2013-2014 period (Petcu et al., 2014), another research was carried out on winter barley on the reduction of the thousand kernel weight as well as on the cuticular transpiration and the stress tolerance index, following the application of potassium iodide. Ongom et al. (2016) also obtained good results which suggested that the stress technique based on desiccants could be used in the sorghum breeding domain.

Barley grain size can be reduced by lack of precipitation and high temperature after anthesis and to improve end-use efficiency (Wang et al., 2021), it has to enhance the barley seed size because this quality parameter correlates with protein and starch content (Yu et al., 2017). The exposure of a barley plant to a high temperature (35^0C) reduces the conversion of sucrose to starch by less than 30% more than a barley plant grown under normal conditions during the grain-filling stage (Wallwork et al., 1998).

Kand et al. (2009) experimented with 148 barley cultivars under high temperatures during the grain-filling period and observed that both protein and starch synthesis were affected. The starch from seeds is the first largest chemical component which counts approximately 60-80% and protein is the second component from the barley grain and could reach from 8 to 20% (Evers et al., 1999).

Therefore, assessing genotype x environment interaction using some parametric and non-parametric stability indices can help to identify barley varieties with superior behavior across growing conditions (Pour-Aboughadareh et al., 2023).

The purpose of this testing was to characterize some winter barley genotypes from the point of view of drought tolerance with reference to the one thousand kernel weight, the stability of the quality indices, i.e., protein and starch content, the size of the seeds, as well as the identification of sources valuable for the translocation of assimilates in limited water conditions for use as parents in the winter barley breeding program.

MATERIALS AND METHODS

In order to evaluate grain quality parameters of barley variety and lines under drought stress, an experiment in the winter barley experimental field of NARDI Fundulea (16 genotypes) during the two seasons (2014-2015 and 2015-2016 period) was conducted. The same genotypes were included in this experiment as checks and treatments. Using a 5-liter hand-held pressure pump sprayer, potassium iodide (0.4% concentration) was applied to the 16 winter barley genotypes (varieties and perspective lines) on 2 rows of 1 m length after 2 weeks from anthesis as the stage of plant development, respecting the differences between the genotypes regarding the flowering date. The neighboring rows were shielded to avoid applying the desiccant on the other rows and to be able to spray the plant up to the flag leaf inclusive. At full maturity, the rows treated with desiccant and the control rows were manually harvested and threshed, after which the mass of 1000 grains (TKW) with the Contador grain counting machine, the protein (P%) and starch (S%) content with the Infratech 1241 NIR analyzer were determined.

The size of barley seeds (plump grains or assortment I and II expressed in %) was measured using one hundred seeds grams (± 0.1 g) of each sample (two replications) which was passed through three consecutive sieves (> 2.8 mm, > 2.5 mm, > 2.2 mm) using a sieve shaker (Sortimat) for 3 minutes (± 10 s). The sample collected in each sieve was weighed, the percentages were recorded and only the 2.8 mm and 2.5 mm were used in this analysis (the seeds used in the malting process).

The percentage of reduction in the case of TKW and assortment I (seeds > 2.8 mm) and increase in the values of the analyzed parameters (protein, starch, and assortment II - seeds > 2.5 mm) was determined based on the formula:

% KI = (Gc-Gd)/Gc*100 (Petcu et al., 2014),

where: % KI - the percentage of KI reduction, Gc - control weight, Gt - treated weight.

The obtained values for each index were analyzed with Statistical Analysis Step-By-Step Using a Statistical Calculator (Dhakre and Bhattacharya, 2018), and the stability of the quality indices at the grain level using the STABILITYSOFT program (Pour-Aboughadareh et al., 2019).

Two statistical parameters were obtained namely regression coefficient (b_i - Finlay and Wilkinson, 1963) and coefficient of variance (CVi - Francis and Kannenberg, 1978) to assess genotype stability by relating genotypic responses (TKW, protein and starch content, seed size) to environmental conditions (rainfall stress due to desiccant), and one non-parametric Kang's rank-sum (*KR* - Kang, 1988) which can explain environment and phenotype relative to the limited water condition (abiotic factor).

RESULTS AND DISCUSSIONS

The desiccant inhibits photosynthesis and the ability of the genotype to fill the grains with the reserves in the stem is manifested.
Within a set of 16 winter barley genotypes (varieties and perspective lines), the variation of some quality parameters was caused by using a desiccant called potassium iodide (KI-0.4%), which led to the characterization of the indices of grain quality, namely thousand kernel weight (TKW), protein content, starch, and grain size (assortment I and II, 2.8 and 2.5 mm).
The analysis of the obtained results revealed significant differences, regarding the influence of the genotype and the applied treatment on TKW and the treatment on the starch content (Table 1).

Table 1. The value of probability and significance (TKW, protein, and starch content)

Check	TKW		Protein		Starch	
Source	p-value	sign.	p-value	sign.	p-value	sign.
Genotypes	0.004	** 1%	0.120	ns	0.648	ns
Replications	0.000	** 1%	0.600	ns	0.317	ns
Treatment	TKW		Protein		Starch	
Source	p-value	sign.	p-value	sign.	p-value	sign.
Genotypes	0.035	* 5%	0.182	ns	0.738	ns
Replications	0.000	** 1%	0.563	ns	0.009	** 1%

*, **significant for p = 5% and p = 1%, ns - nonsignificant

Table 2. The value of probability and significance (assortment I and II, 2.8 and 2.5 mm)

Check	Assortment I		Assortment II	
Source	p-value	sign.	p-value	sign.
Genotypes	0.0001	** 1%	0.0060	** 1%
Replications	0.0001	** 1%	0.1960	ns
Treatment	Assortment I		Assortment II	
Source	p-value	sign.	p-value	sign.
Genotypes	0.0007	** 1%	0.0006	** 1%
Replications	0.0084	** 1%	0.0060	** 1%

*, **significant for p = 5% and p = 1%, ns - nonsignificant

In the case of grain size (Table 2), the influence of genotype was significant for both 2.8 mm and 2.5 mm grain sizes. The analysis carried out showed significant differences between the genotypes, thus, in the case of TKW, the barley varieties with six rows of grains Dana, Univers, Ametist, Lucian, and the F 8-10-12 line were noted, as well as the varieties with two rows of grains Artemis and Gabriela (Table 3) with the highest values**.**
The TKW parameter showed a reduction from 1.3% (Smarald variety) to 10.4% (DH 267-66 line) and it has to be underlined 9 genotypes registered values over 42.0 g (Table 3).
The grain protein and starch deposition are limited by water availability during the growing season. Under limited water growing conditions, the converted sucrose quantity to starch is reduced and therefore the TKW but at the same time, the protein was diluted by starch according to Emebiri et al., 2001.
Under the same test conditions, the protein content and the starch content did not decrease below 9.5% and 60%, respectively (standards required by the malt and beer industry). Regarding assortment I (Table 4), in every studied case this parameter (> 2.8 mm seed size) showed a reduction between 1.3% (six-row Dana variety) and 40.6% (two-row DH 267-66 line).
The smallest reduction (from 41.7 to 37.8%) of the six-row Lucian variety (9.4%) compared with the highest (41.4%) for the six-row F 8-10-12 line was registered by the assortment II (> 2.5 mm seed size).
The regression coefficient value of individual genotypes was suggested by Finlay and Wilkinson (1963) as the response to the favourability/unfavorability of the plant-growing environment.
There are three situations: the genotype is well adapted to all growing environments when this coefficient is almost 1 or does not differ from 1 (the regression intercept is large) and when the $bi \geq 1$ (the regression intercept is large), the genotype is widely adapted). A $bi > 1$ shows that genotypes have good behavior in favorable environments, whereas a $bi < 1$ describes good adaptability in unfavorable environments (the intercept is large. According to these, a characterization of the studied parameters was performed.
Regarding the reduction or/and increase in seed quality parameters and size under drought conditions, a barley genotype can be characterized as adapted to unfavorable environments when the regression coefficient $b < 1$ and the regression intercept is large. When $b > 1$ the barley variety is adapted to favorable

Table 3. Experimental data comparison before and after the desiccant treatment (TKW, protein, and starch content)

No.	Genotypes	TKW (g)		Reduction (%)	Protein (%)		Reduction/ Increase (%)	Starch (%)		Reduction/ Increase (%)
		Check	Treatment		Check	Treatment		Check	Treatment	
1	Dana	46.7	43.9	5.9	12.3	11.9	-3.3	61.5	61.9	-0.73
2	Cardinal	39.8	38.1	4.2	10.8	10.2	-5.6	62.9	63.0	-0.24
3	Univers	46.9	42.2	10.0	10.8	11.0	+1.9	62.8	62.6	+0.32
4	Ametist	46.4	44.6	3.8	11.4	11.7	+2.6	62.4	61.5	+1.44
5	Smarald	39.4	39.4	1.3	11.5	11.4	-0.4	62.8	63.0	-0.40
6	Simbol	44.0	39.8	9.5	11.5	11.2	-2.6	62.9	62.8	+0.16
7	F8-19-10	42.7	39.0	8.7	10.7	10.5	-2.3	63.3	62.9	+0.63
8	F8-3-01	39.2	38.5	1.8	11.5	11.6	+0.9	62.6	61.9	+1.04
9	Lucian	43.4	41.5	4.5	12.3	12.6	+2.0	62.6	62.0	+0.96
10	Onix	43.3	40.5	6.4	12.2	12.0	-2.0	62.8	63.0	-0.32
11	F8-10-12	48.5	46.0	5.2	12.0	12.2	+1.7	61.5	61.7	-0.24
12	Andreea	45.7	43.0	6.0	12.7	12.5	-1.6	62.8	62.2	+0.96
13	Artemis	51.2	48.0	6.3	12.8	12.4	-2.7	62.3	62.2	+0.16
14	DH267-66	47.5	42.6	10.4	11.5	11.8	+3.1	63.2	62.9	+0.48
15	Gabriela	52.8	48.6	8.0	13.3	12.6	-5.3	61.8	62.2	-0.65
16	DH315-10	47.1	45.3	3.8	14.0	13.4	-4.3	62.5	62.5	0.00
Mean		45.2	42.5	6.0	11.9	11.8	2.6	62.5	62.3	
R^2		0.91	0.91		0.65	0.62		0.47	0.57	
CV (%)		6.02	6.77		8.03	7.87		1.34	1.35	
LSD (5%)		5.56	5.87		1.95	1.89		1.71	1.72	

Table 4. Experimental data comparison before and after the desiccant treatment (assortment I and II)

No.	Genotypes	Assortment I (> 2.8 mm)		Reduction (%)	Assortment II (> 2.5 mm)		Reduction /Increase (%)
		Check	Treatment		Check	Treatment	
1	Dana	44.8	44.2	-1.3	36.9	38.2	+3.5
2	Cardinal	54.8	47.5	-13.4	28.1	34.7	+23.5
3	Univers	66.9	58.4	-12.7	24.0	25.8	+7.5
4	Ametist	67.1	47.7	-28.9	25.6	31.2	+21.9
5	Smarald	49.3	47.7	-3.2	25.9	28.4	+9.7
6	Simbol	45.9	26.2	-42.9	34.8	40.3	+15.8
7	F8-19-10	32.8	29.5	-10.0	41.9	35.7	-14.8
8	F8-3-01	42.2	33.6	-20.4	32.4	36.8	+13.6
9	Lucian	43.5	33.5	-23.0	41.7	37.8	-9.4
10	Onix	58.3	39.5	-32.3	31.4	39.7	+26.4
11	F8-10-12	65.2	40.4	-38.1	26.8	37.9	+41.4
12	Andreea	52.5	37.5	-28.7	38.5	47.8	+24.2
13	Artemis	75.4	67.3	-10.7	19.6	20.4	+4.1
14	DH267-66	50.3	29.9	-40.6	39.7	43.8	+10.3
15	Gabriela	86.2	66.0	-13.5	9.5	23.8	+22.7
16	DH315-10	66.2	58.7	-11.3	24.9	31.3	+25.7
Mean		35.51	65.05		37.82	18.19	
R^2		0.89	0.92		0.87	0.80	
CV (%)		26.36	9.18		19.23	26.86	
LSD (5%)		19.09	12.17		14.83	9.96	

Table 5. Variation of coefficient of regression (b_i), coefficient of variation (CVi), and Kang's rank-sum (KR) of TKW, protein, and starch content

Genotype	TKW (g)	b_i	CVi	*K*R	P (%)	b_i	CVi	*K*R	S (%)	b_i	CVi	*K*R
Dana	45.3	1.010	4.29	4	12.1	2.618	2.63	7	61.7	-2.370	0.45	15
Cardinal	38.9	0.606	2.99	14	10.5	3.782	4.38	16	63.0	-0.593	0.11	6
Univers	44.6	1.727	7.46	10	10.9	-1.164	1.29	15	62.7	1.481	0.28	6
Ametist	45.5	0.643	2.72	8	11.6	-1.745	1.83	13	62.0	5.333	1.02	15
Smarald	39.4	0.050	0.60	15	11.5	0.582	0.61	6	62.9	-1.185	0.22	8
Simbol	41.9	1.543	7.08	13	11.3	2.036	2.18	10	62.8	0.889	0.16	3
F8-19-10	40.9	1.359	6.40	11	10.6	1.455	1.67	12	63.1	2.370	0.44	3
F8-3-01	38.8	0.257	1.27	15	11.5	-0.291	0.30	9	62.3	4.148	0.79	13
Lucian	42.4	0.716	3.25	9	12.4	-1.455	1.42	8	62.3	3.556	0.68	11
Onix	41.9	1.010	4.64	7	12.1	1.455	1.46	1	62.9	-0.889	0.16	8
F8-10-12	47.2	0.918	3.74	3	12.1	-1.164	1.16	4	61.6	-0.889	0.17	12
Andreea	44.3	1.010	4.38	4	12.6	1.164	1.12	3	62.5	3.556	0.67	10
Artemis	49.6	1.176	4.56	2	12.6	2.327	2.24	5	62.3	0.593	0.11	1
DH267-66	45.0	1.819	7.77	11	11.7	-1.745	1.82	13	63.0	2.074	0.39	3
Gabriela	50.7	1.543	5.86	1	12.9	4.364	4.10	10	62.0	-2.074	0.39	14
DH315-10	46.2	0.661	2.75	6	13.7	3.782	3.36	2	62.5	0.055	0.15	2

Table 6. Variation of b_i (coefficient of regression), CVi (coefficient of variation), and KR (Kang's rank-sum) of grain size (assortment II and I)

Genotype	S I (%)	b_i	CVi	*K*R	S II (%)	b_i	CVi	*K*R
Dana	44.5	0.047	0.90	15	37.6	0.296	2.50	5
Cardinal	51.1	0.607	10.16	6	31.4	1.463	14.80	7
Univers	62.7	0.699	9.55	1	24.9	0.390	4.97	13
Ametist	57.4	1.599	23.85	6	28.4	1.254	14.01	6
Smarald	48.5	0.132	2.33	12	27.1	0.550	6.43	11
Simbol	36.1	1.624	38.56	14	37.5	1.220	10.32	2
F8-19-10	31.2	0.272	7.49	16	38.7	-1.370	11.23	12
F8-3-01	37.9	0.712	16.08	10	34.6	0.973	8.93	2
Lucian	38.5	0.824	18.32	6	39.7	-0.880	7.03	7
Onix	48.9	1.556	27.27	9	35.5	1.844	16.47	10
F8-10-12	52.8	2.047	33.21	11	32.4	2.478	24.31	13
Andreea	45.0	1.242	23.66	4	43.2	2.078	15.28	4
Artemis	71.4	0.666	7.99	1	20.0	0.174	2.75	15
DH267-66	40.1	1.686	36.01	13	41.8	0.917	6.97	1
Gabriela	76.1	1.671	18.82	4	16.6	3.180	60.68	16
DH315-10	62.5	0.617	8.45	3	28.1	1.432	16.17	7

conditions and when $b \geq 1$ and the regression intercept is large, the variety is widely adapted. The most desirable genotypes are considered the genotypes with low environmental variance and with a low coefficient of variation (CVi) as suggested by Francis and Kannenberg (1978). Kang's rank-sum (Kang, 1988) is a parameter that gives a weight of 1 to both yield and stability statistics in order to identify high-yielding and stable genotypes and uses both yield and intercepts as selection criteria. The genotype with the highest yield and lower intercepts is assigned a rank of 1, the ranks are added for each genotype and those with the lowest rank-sum are the most desirable.

The studied winter barley varieties and advanced lines from this experiment varied widely in the TKW (0.050 Smarald variety and 1.819 DH 267-66 line), protein content (0.582 Smarald variety and 4.364 Gabriela variety), and starch content (0.593 Artemis variety and 5.333 Ametist variety) regression coefficient (Table 5), while the CVi was low (for TKW from 0.60 to 7.08, for protein content from 0.30 to 4.10 and for starch content from 0.11 to 1.02). A coefficient of variation below 10 shows very good stability of the parameters under drought conditions. The KR sum showed a good environment and phenotype relative to the abiotic-induced factor for Gabriela, Artemis, F 8-10-12, Andreea, and Dana genotypes regarding TKW. The lowest protein rank sum was registered by the Onix, DH 315-10, Andreea, F 8-10-12, and Artemis genotypes while for starch content the best genotypes were Artemis, DH 315-10, DH 267-66, Simbol, and F 8-19-10 (Table 5).

The grain size regression coefficient also widely varied (both sizes) and the CVi of assortment I ranged between 2.33 (Smarald variety) and 38.56 (Simbol variety), only six genotypes registered a CVi below 10 while a number of sixteen between 10,16 and 38.56 (Table 6).

The genotype stability by relating genotypic responses was different regarding assortment II due to a higher variation of CVi (2.75-60.68) and it is observed that some genotypes had a lower CVi than in the case of assortment I meaning a higher percent of grain >2.5 mm. Regarding the KR sum for assortment I (Table 6), the best genotypes are Univers, Artemis, Gabriela, DH 315-10, and Andreea while for assortment II, the DH 267-66, F 8-3-01, Simbol, and Andreea.

CONCLUSIONS

Comparing parameter values, among tested genotypes, some of them were sensitive and others were tolerant to water-limiting conditions.

Significant results regarding the identification of valuable genetic resources for the translocation of assimilates under water-limiting conditions, for use as parents in the breeding program, as well as the characterization of varieties and lines with reference to the high stability of grain weight, were obtained.

These winter barley genotypes have a high content of carbohydrates in the stem and leave at the beginning of the grain filling period, but also a high rate of translocation to seed. Furthermore, for the varieties and lines with high stability of grain weight (TKW), it is necessary to study the individual contribution of the stem, leave, and spike and also in order to establish if the spike partially compensates the photosynthesis through palea and awns.

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TANK MIXTURE OF PLANT PROTECTION PRODUCTS WITH BIOSTIMULANT IN WINTER RYE (*Secale cereale* L.)

Mariyan YANEV[1], Nesho NESHEV[1], Anyo MITKOV[1], Todor MANILOV[1], Maya DIMITROVA[1], Cveta MOSKOVA[2]

[1]Agricultural University of Plovdiv, 12 Mendeleev Blvd, Plovdiv, Bulgaria
[2]University of Forestry, 10 Kliment Ohridski Blvd, Sofia, Bulgaria

Corresponding author email: mar1anski@abv.bg

Abstract

The application of various chemical products for plant protection and plant biostimulans increases financial costs, which is a prerequisite for their combined application in tank mixtures. During 2021 and 2022 on the experimental field of the Agricultural University of Plovdiv, Bulgaria, a field experiment with the rye variety "Milenium" was performed. The aim of the trial was to study the application of plant protection products in tank mixture with biostimluant and its influence to the rye grain yield and quality. The trial included the following treatments: 1. Untreated control; 2. Granstar 75 DF - 15 g ha^{-1} (herbicide); 3. Granstar 75 DF - 15 g ha^{-1} + Zantara 216 EC - 1.25 l ha^{-1} (fungicide); 4. Granstar 75 DF - 15 g ha^{-1} + Amino Expert Impuls - 3.00 l ha^{-1} (biostimulant); 5. Granstar 75 DF - 15 g ha^{-1} + Zantara 216 EC - 1.25 l ha^{-1} + Amino Expert Impuls - 3.00 l ha^{-1}. The highest rye grain yield, absolute and hectoliter seed mass, grain wet gluten and crude protein for treatment 5 were recorded.

***Key words**: rye, yield, quality, pesticides, tank mixture.*

INTRODUCTION

The progressive growth of the population worldwide necessitates an increase in the food production. This necessitates the use of new technologies that minimize problems caused by pests, nutrient deficiencies, competition between plants, water shortages, etc. (Grzebisz, et al., 2022; Georgiev et al., 2019; Shopova & Cholakov, 2015; Shopova and Cholakov, 2014; Bernstein et al., 2011; Calkins and Swanson, 1995). The use of different chemicals to control pests in cultivated areas has high operating costs and therefore their combined application is often applied by farmers (Arru et al., 2012). Approximately 97% of farmers mix 6 or more products which are simultaneously applied (Gazziero, 2015). Surfactants are typical of these mixtures and these products can induce synergistic effects in pest control (Li et al., 2019).

Winter cereal crops occupy a major share of cultivated areas not only in our country, but also abroad.

Mixing pesticides in a tank mix results in lower production costs. The combined application reduces the number of machine entries into the cultivated land, fuel consumption, water use for solution preparation and hours spent, resulting in less compaction of the soil (Gazziero, 2015). However, tank mixtures of pesticides pose environmental challenges as they can cause production loss as well as environmental risks such as cross-source pollution (Vale et al., 2019) and neurotoxic effects on living organisms (Wang et al., 2015). They can undergo three types of interactions (Ikeda, 2013): 1. Enhancing - when the efficacy of the product mix is similar to the efficacy of each product individually; 2. Synergistic - when the tank mixture of certain products gives better results than the application of each separately; 3. Antagonistic - when the result of the products applied in a tank mixture is worse than each individually.

Herbicidal products are widely used in agriculture. Most herbicide mixtures used to control certain weeds, such as those that are tolerant or even resistant to a particular product, have an additive effect. Mixtures of different types of agrochemicals can have antagonistic effects because they include more than one category of substances used to control pests. There is evidence that the use of insecticides or fungicides together with herbicides in tank mixtures can reduce the selectivity of the preparations to crop replantation (Gassen, 2002). Mixtures of different groups of pesticides can also affect plant metabolism (Hartzler et al., 2000). Mixtures of insecticides and herbicides can be toxic to natural bioagents, as for example an increase in *T. podisi* intoxication has been reported following the combined application of the insecticides cypermethrin, thiamethoxam and bifenthrin together with the herbicides cyhalofop-butyl, imazethapyr, imazapic and penoxulam (Pazini et al., 2017).

In recent years, the question of the effect of mixing plant protection products and biostimulants and their use in a tank mixture has been increasingly raised. There is very little scientific research on this issue, relying mainly on data from the companies that offer them. However, these are studies done abroad, with different combinations of plant protection products, depending on the problems posed by practice in the respective countries. It is extremely important to shed light on these questions regarding which component of the mixture has a negative effect on plants, which requires monitoring physical indicators when mixing different plant protection products and foliar fertilizers, biological and physiological indicators that take into account their effect mainly in terms of the cultivated plant. This also defines the aim of the present study.

MATERIALS AND METHODS

The research was conducted in 2020-2022 at the experimental field of the Department of Agriculture and Herbology at the Agricultural University - Plovdiv, Bulgaria with rye, variety "Millennium". The study was based on the method of long plots on an area of 100 m^2.

The following variants were under evaluation:

1. Untreated control
2. Granstar 75 DF - 15 g ha^{-1};
3. Granstar 75 DF - 15 g ha^{-1} + Zantara 216 EC - 1.25 l ha^{-1};
4. Granstar 75 DF - 15 g ha^{-1} + Amino Expert Impuls - 3.00 l ha^{-1};
5. Granstar 75 DF - 15 g ha^{-1} + Zantara 216 EC - 1.25 l ha^{-1} + Amino Expert Impuls - 3.00 l ha^{-1}.

Studied plant protection products and product with biostimulant mode of action:

Herbicide: Granstar 75 DF (750 g/kg tribenuron-methyl);

Fungicide: Zantara 216 EC (50 g/l *bixafen* + 166 g/l tebuconazole);

Plant biostimulant: Amino Expert Impuls (Amino acids, phytohormones, nitrogen, magnesium, sulfur, boron, copper, iron, manganese, molybdenum, zinc);

The application of plant protection products with the biostimulant is in a tank mixture with a volume of the working solution of 210 l ha^{-1}.

The treatment was carried out at the end of March, in the phenophase of the end of tillering - the beginning of spindleing of the crop (BBCH 29-31).

The following indicators were studied:

- Absolute seed mass (g), (Dimitrova et al., 2006);
- Hectoliter seed mas (kg) (Dimitrova et al., 2006);
- Seed crude protein content (%) (By Kjeldahl's method, Tomov et al., 2009);
- Seed wet gluten conent (%) (ISO 21415-2:2015).
- Rye grain yields (t ha^{-1}). The harvest was carried out with a Wintersteiger® field trial harvester.

Levels of phytotoxicity for the crop by the 9-score scale of EWRS (European Weed Research Society) were reported (at score 1 - there is no visible damages to the crop, and at score 9 - the crop plants are completely destroyed). The evaluations were done four times - on the 7^{th}, 14^{th}, 28^{th}, and 56^{th} day after treatments.

In both experimental years the winter rye was grown as a monoculture.

The tillage carried out before rye's sowing was deep plowing, followed by harrowing. Before sowing, fertilization with 250 kg ha^{-1} with NPK 15:15:15 and spring dressing with 250 kg ha^{-1} NH_4NO_3 was accomplished.

Duncan's method with the SPSS 19 program (Duncan, 1955) was used for the statistical processing of the obtained data. Differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSIONS

Table 1 shows the amounts of precipitation during the rye's growing seasons (2020/2021 and 2021/2022). The precipitations measured are a prerequisite for relatively good moisture storage and normal vegetation during both experimental years. Rye was sown in October, with germination and emergence taking place at high soil moisture. During the vegetation periods, no water deficit is observed.

Table 1. Average monthly precipitation (mm) and average monthly minimum and maximum temperatures during the vegetation seasons of rye (°C)

Months	2020/2021			2021/2022		
	Precipitation, mm	Average monthly temperature, min. t°	Average monthly temperature, max. t°	Precipitation, mm	Average monthly temperature, min. t°	Average monthly temperature, max. t°
October	62.3	9.2	22.3	180.0	9.0	12.6
November	50.7	1.6	13.3	26.5	6.0	11.4
December	51.8	2.4	9.0	124	0.4	8.1
January	29.3	-1.4	10.1	48.8	0.8	5.8
February	32.8	-0.7	18.4	58.8	3.5	5.6
March	43.3	0.8	13.9	76.0	1.0	13.5
April	67.4	4.8	18.8	52.0	10.7	16.9
May	58.1	11.4	26.5	35.5	14.1	24.2
June	51.7	15.3	30.9	106.8	20.8	26.1

Temperatures (min. and max.) were favourable for plant development as well. Despite the high winter temperatures, no negative influence of the warm winter months on the growth and development of the crop plants was found. The analysis of meteorological data shows that the experimental years are favourable for the growth, development and realization of the productive possibilities of rye. During the two experimental years, the selectivity of the applied products to the crop was also studied. Under the conditions of the experiment and during the four reporting dates of the two years, no visible symptoms of phytotoxicity were found after all treatments - score 1 by the EWRS scale.

Table 2 presents the results of the rye grain yield, the absolute and hectoliter seed mass.

Table 2. Rye grain yield (t ha^{-1}), absolute seed mass (g) and hectoliter seed mass (kg)

Treatments	Grain yields (t ha^{-1})		
	2021	2022	Average
1. Untreated control	2.36 c	2.24 d	2.30
2. Granstar 75 DF - 15 g ha^{-1}	3.21 b	3.06 c	3.14
3. Granstar 75 DF - 15 g ha^{-1} + Zantara 216 EC - 1.25 l ha^{-1}	3.25 b	3.12 c	3.19
4. Granstar 75 DF - 15 g ha^{-1} + Amino Expert Impuls - 3.00 l ha^{-1}	3.29 ab	3.32 b	3.31
5. Granstar 75 DF - 15 g ha^{-1} + Zantara 216 EC - 1.25 l ha^{-1} + Amino Expert Impuls - 3.00 l ha^{-1}	3.38 a	3.42 a	3.29
Treatments	Absolute seed mass (g)		
	2021	2022	Average
1. Untreated control	26.07 e	25.26 c	25.67
2. Granstar 75 DF - 15 g ha^{-1}	29.23 b	26.37 b	27.80
3. Granstar 75 DF - 15 g ha^{-1} + Zantara 216 EC - 1.25 l ha^{-1}	27.47 c	26.20 b	26.84
4. Granstar 75 DF - 15 g ha^{-1} + Amino Expert Impuls - 3.00 l ha^{-1}	29.27 b	26.34 b	27.81
5. Granstar 75 DF - 15 g ha^{-1} + Zantara 216 EC - 1.25 l ha^{-1} + Amino Expert Impuls - 3.00 l ha^{-1}	30.67 a	27.40 a	29.04
Treatments	Hectoliter seed mass (kg)		
	2021	2022	Average
1. Untreated control	65.67 d	64.33 c	65.00
2. Granstar 75 DF - 15 g ha^{-1}	66.67 de	65.67 b	66.17
3. Granstar 75 DF - 15 g ha^{-1} + Zantara 216 EC - 1.25 l ha^{-1}	67.33 cd	66.00 b	66.67
4. Granstar 75 DF - 15 g ha^{-1} + Amino Expert Impuls - 3.00 l ha^{-1}	68.67 b	67.16 ab	67.92
5. Granstar 75 DF - 15 g ha^{-1} + Zantara 216 EC - 1.25 l ha^{-1} + Amino Expert Impuls - 3.00 l ha^{-1}	70.00 a	67.83 a	68.92

Figures with different letters are with a proven difference by Duncan's multiple range test ($p < 0.05$).

After the application of herbicides and biostimulants, Matysiak et al. (2018) found an increase in wheat grain yield. The highest grain yields in the current trial in option 5 were recorded (after the combined applicacion in a tank mixture of Granstar 75 DF - 15 g ha^{-1} + Zantara 216 EC - 1.25 l ha^{-1} + Amino Expert Impuls - 3.00 l ha^{-1}) - 3.29 t ha^{-1}, both in the two experimental years and on average over the study period. These data were statistically proven. The lowest yields were obtained in the untreated control.

The absolute seed mass is of great importance for yields (Georgiev et al., 2014).

The absolute seed mass differed among the treatments. In the first experimental year, in relation to this indicator, there were proven differences between the treated variants, as well as with the untreated control, while in the second year the absolute seed mass of treatments 2, 3 and 4 showed close values that were not statistically significant.

The highest absolute seed mass for variant 5 was obtained (Granstar 75 DF - 15 g ha^{-1} + Zantara 216 EC - 1.25 l ha^{-1} + Amino Expert Impuls - 3.00 l ha^{-1}) - 29.04 g on average for the period, and the lowest - in the untrained control was recorded - 25.67 g.

As both yields and absolute seed mass, the hectoliter mass was affected by the treatments as well.

The highest hectoliter seed mass for the rye seeds of variant 5 was measured (Granstar 75 DF - 15 g ha^{-1} + Zantara 216 EC - 1.25 l ha^{-1} + Amino Expert Impuls - 3.00 l ha^{-1}) - 68.92 kg , and this indicator was the lowest for the control - 65.00 kg.

Crude protein content varies depending on the variety and is affected by nitrogen fertilization (Bártová et al., 2013). The highest content of this indicator was reported in the seeds at treatment 5 (Granstar 75 DF - 15 g ha^{-1} + Zantara 216 EC - 1.25 l ha^{-1} + Amino Expert Impuls - 3.00 l ha^{-1}) - 13.99 %, average for the experimental conditions, while in the control the lowest crude protein content was 10.97% recorded. In treatments 2 and 3 values were approximately similar in the different years and were not statistically proven.

Xhaferaj et al. (2023) examined samples of 32 rye varieties, and the results showed that the gluten content of rye flour varied from 3.0 to 7.8 g/100 g. The data from the present experiment showed that this indicator of the rye, variety “Millennium”, varies from 5.07 to 7.78%. The lowest results were found for the untreated control and were reliable compared to the treated variants (2, 3, 4 and 5). The highest results for this indicator were obtained for treatment 5 (Granstar 75 DF - 15 g ha^{-1} + Zantara 216 EC - 1.25 l ha^{-1} + Amino Expert Impuls - 3.00 l ha^{-1}) - 7.78%.

Table 3. Rye grain crude protein (%) and wet gluten (%)

Treatments	Crude protein (%)		
	2021	2022	Average
1. Untreated control	11.15 c	10.79 c	10.97
2. Granstar 75 DF - 15 g ha^{-1}	13.47 b	12.53 b	13.00
3. Granstar 75 DF - 15 g ha^{-1} + Zantara 216 EC - 1.25 l ha^{-1}	13.24 b	12.76 b	13.00
4. Granstar 75 DF - 15 g ha^{-1} + Amino Expert Impuls - 3.00 l ha^{-1}	14.09 ab	13.40 a	13.75
5. Granstar 75 DF - 15 g ha^{-1} + Zantara 216 EC - 1.25 l ha^{-1} + Amino Expert Impuls - 3.00 l ha^{-1}	14.28 a	13.69 a	13.99
Treatments	Wet gluten (%)		
	2021	2022	Average
1. Untreated control	5.60 c	4.54 c	5.07
2. Granstar 75 DF - 15 g ha^{-1}	7.50 a	7.83 a	7.67
3. Granstar 75 DF - 15 g ha^{-1} + Zantara 216 EC - 1.25 l ha^{-1}	7.10 ab	6.26 b	6.68
4. Granstar 75 DF - 15 g ha^{-1} + Amino Expert Impuls - 3.00 l ha^{-1}	6.90 b	7.37 a	7.14
5. Granstar 75 DF - 15 g ha^{-1} + Zantara 216 EC - 1.25 l ha^{-1} + Amino Expert Impuls - 3.00 l ha^{-1}	7.74 a	7.81 a	7.78

Figures with different letters are with a proven difference by Duncan's multiple range test ($p < 0.05$).

CONCLUSIONS

After the treatment of the rye with the herbicide Granstar 75 DF - 15 g ha^{-1} alone, or in combination with the fungicide Zantara 216 EC - 1.25 l ha^{-1} or with the amino stimulant Amino Expert Impuls - 3.00 l ha^{-1}, no visible symptoms of phytotoxicity were detected on the crop. The highest yields, absolute and hectoliter mass of the seeds, crude protein and gluten content were reported for variant 5 (tank mixture of Granstar 75 DF - 15 g ha^{-1} + Zantara 216 EC - 1.25 l ha^{-1} + Amino Expert Impuls - 3.00 l ha^{-1}), which are statistically proven, compared to the untreated control.

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INFLUENCE OF FERTILIZATION ON SOME PARAMETERS OF GROWTH AND DEVELOPMENT OF *Triticum monococcum* L. IN THE CONDITIONS OF ORGANIC FARMING IN BULGARIA

Yordan YORDANOV, Tonya GEORGIEVA, Malgozhata BEROVA

Agricultural University of Plovdiv, 12 Mendeleev Blvd, Plovdiv, Bulgaria

Corresponding author email: j.jordanov72@gmail.com

Abstract

The purpose of the study is to determine the influence of fertilization on the growth and development of Triticum monococcum L. A three-year field experiment was conducted at the Agricultural University - Plovdiv. A block method was used in four replications, with a plot area of 15 m^2. The main factors of the study are: year (2018-2019; 2019-2020 and 2020-2021), and fertilization (Control - no fertilization; Italpolina - soil fertilizer, Naturamin WSP - foliar fertilizer). The phenological development of the crop was analyzed, as well as interphase periods. The growing season was 231 days in which they accumulated 1512.2°C effective temperature sums. Growth in height is highly dependent on the year. Plants reach sizes of 81.19 to 113.18 cm. The year has a strong influence on tillering - the most number of tillers per plant (4.4-4.6) develop in the second and third years. Italpolina - soil fertilizer has a better effect on this indicator. The influence of soil fertilizer is stronger compared to foliar fertilizer both on the number of productive tillers/plant and on the number of productive stems/m^2.

Key words: *Triticum monococcum L., growth, phenophases, fertilization, organic farming.*

INTRODUCTION

A number of authors consider *Triticum monococcum* L. to be the most ancient cereal crop, which is believed to have been used for human food as early as 10.000 years ago (Salamini et al., 2002). According to Arnaudov (1936a; 1936b) and Lipshits (1945), this species was cultivated in the Thracian lands more than 3500 years ago. According to Demosten (384-322 BC), grains of *Triticum monococcum* L. were found in Thracian underground storages (Katzarov, 1912; Ikonom, 1936). It was called "the bread of the Thracians", "the wheat of the pharaohs", "the last food of Christ". In German and English it is known as "einkorn", in French "engrain", in Hungarian "alakor", and in Russian as "odnosrianka" (Stamatov et al., 2017). In Bulgaria, Stranski (1929) studied einkorn and described 4 wild and 11 domesticated varieties of the species, including some endemic ones (var. *bulgaricum,* var. *sofianum*).

Until 40 years ago, einkorn production was limited to small isolated regions in France, India, Italy, Turkey and the former Yugoslavia (Harlan, 1981; Perrino and Hammer, 1984).

Currently, there is a renewed interest in this crop due to the nutritional qualities of the grain, its adaptation to economic cultivation and high resistance to diseases and enemies, which are an advantage for organic farming (Stamatov et al., 2012). Cultivated einkorn is also a valuable source of genes for the breeding improvement of modern wheats (Zaharieva and Monneveux, 2014).

Today, einkorn can be found all over Bulgaria, but more often as a weed. It is grown in small areas in the area of the cities of Haskovo, Stara Zagora, Yambol, Pernik, Sofia and Kyustendil.

According to the Bulgarian scientist Stranski (1929, 1934), the einkorn was brought by the ancient Bulgarians to the lands of the former Volga-Kam Bulgaria from the Balkan Peninsula, and the German researcher Schiemann (1954) determined the distribution directory of einkorn, which started from the Bulgarian lands and went in the Volgo-Kama-Ural direction. According to Arnaudov (1936a), this plant was found during archaeological excavations near the village of Veselinovo (Yambol region), in the form of charred mature classes.

The large number of wild and cultivated varieties of this species in these lands is an indication that Bulgaria is a secondary formative center.

Current trends towards sustainable and economical agriculture, as well as the use of "biological" and "functional" foods, suggest that this cereal can play a significant role in the dietary and healthy nutrition of humans. Additional research is needed to specify some components of the technology for organic farming in Bulgaria.
The aim of the present study is to determine the influence of fertilization with products for biological production on phenological development, tillering dynamics, growth in height and the formation of the productive stem.

MATERIALS AND METHODS

To achieve the aim of the study, a field experience was undertaken at the Agroecological Center - Demonstration Center for Organic Agriculture at the Agricultural University - Plovdiv, Bulgaria, in the period 2018 - 2021. The Agroecological Center has been a member of the International Federation of Organic Agriculture (IFOAM) since 1993. A block method was used in four replications, with a harvest plot area of 15 m^2. A common vetch (*Vicia sativa* L. ssp. *sativa*), sown in spring, was used as a preceding crop for the experiment.
The following variants have been tested: Control - without fertilization; Fertilization with soil fertilizer - Italpolina, in a dose of 0.7 t/ha; Fertilization with foliar fertilizer - Naturamin WSP - an amino acid product applied in three treatments - in the tillering, stem elongation and heading phases with 30 g/da. The year was also considered as a factor in the statistical processing for the three-year period.
The seeds are from local forms of einkorn, produced in an organic farm and provided by the Institute of Plant Genetic Resources "K. Malkov" town of Sadovo. They are certified with the relevant necessary documents of origin.
Statistical processing of the experimental data was performed using the SPSS V. 9.0 for Microsoft Windows program, using Duncan's method, Anova.
The soils in the Agroecological Center are alluvial - meadow (Mollic Fulvisols) (FAO-UNESCO, 1988). They have low N availability, low to medium P availability, and good K availability. The soil analyzes (Table 1) made immediately before sowing confirm the previously made characterization of the soil (Popova and Sevov, 2010). The soil conditions in the three years are relatively the same.

Table 1. Agrochemical indicators of the soils in the experimental areas

Year	pH (H_2O)	NH_4^+	NO_3^{2-}	NH_4^+ NO_3^{2-}	P_2O_5	K_2O
		mg/kg	mg/kg	mg/kg	mg/ 100 g	mg/ 100 g
2018	7.3	12.0	19.0	31.0	7.8	32.0
2019	7.5	23.4	16.5	39.9	13.6	29.2
2020	7.8	19.3	8.3	27.6	12.5	33.1

Agrometeorological conditions in the experimental period are presented in Figures 1, 2 and 3.

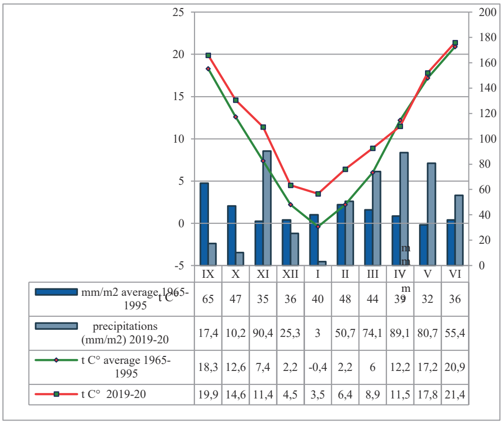
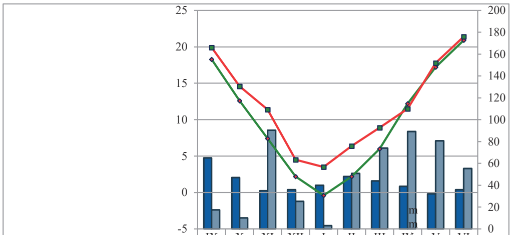


Figure 1. Agrometeorological conditions for the development of *Triticum monococcum* L. in the region of Plovdiv (2018/2019)

The einkorn growing season in 2018/2019 is defined as warm and wet (Figure 1). Temperatures in autumn - September, October and November are higher than the monthly averages for the long-term period. At that time, however, rainfall is limited, which delays the preparation of the soil and the timely entry into the third leaf and tillering phases at an optimal time. The amount of precipitation in the September-June period is about 50 l/m^2 more than the norm, but it is disproportionately distributed. The months of November, April and June are distinguished by the maximum rainfall. Rainfall in the autumn months of 2019 (Figure 2) was more favorable for treatments, the plants passed normally through the third leaf phases and entered tillering on time. Crops are provided with sufficient amounts of moisture.

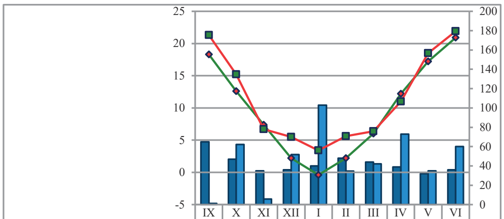
Until ripening, the plants develop in optimal agrometeorological conditions.



	IX	X	XI	XII	I	II	III	IV	V	VI
mm/m2 average 1965-1995	65	47	35	36	40	48	44	39	32	36
precipitations (mm/m2) 2019-20	17,4	10,2	90,4	25,3	3	50,7	74,1	89,1	80,7	55,4
t C° average 1965-1995	18,3	12,6	7,4	2,2	-0,4	2,2	6	12,2	17,2	20,9
t C° 2019-20	19,9	14,6	11,4	4,5	3,5	6,4	8,9	11,5	17,8	21,4

Figure 2. Agrometeorological conditions for the development of *Triticum monococcum* L. in the region of Plovdiv (2019/2020)

The third experimental year (Figure 3) started with temperatures above normal and very little moisture, especially in the month of November, but by the end of the growing season the conditions were favorable for normal plant development.



	IX	X	XI	XII	I	II	III	IV	V	VI
mm/m2 average 1965-1995	65	47	35	36	40	48	44	39	32	36
precipitations (mm/m2) 2020-21	1,3	62,3	5,7	51,8	103	34,6	42,2	72,9	34,9	60
t C° average 1965-1995	18,3	12,6	7,4	2,2	-0,4	2,2	6	12,2	17,2	20,9
t C° 2020-21	21,3	15,2	6,7	5,5	3,4	5,6	6,4	11	18,5	21,9

Figure 3. Agrometeorological conditions for the development of *Triticum monococcum* L. in the region of Plovdiv (2020/2021)

The three years of the study appear to be significantly warmer than normal, which is in line with global trends. For a 6-month growing season, the average monthly temperature is 1.7°C higher than the norm for 2018-2019 (X to VI), 3.2°C higher for the same period in 2019-2020 and by 1.7°C - in the last year of study.

Precipitation in the growing seasons is in larger amounts, but not always evenly distributed, which reflects the duration of the interphase periods and the optimal course of life processes.

RESULTS AND DISCUSSIONS

Phenological development. The vegetation period of einkorn under the experimental conditions was found to be 230 to 233 days (Table 2). It begins with germination between November 5 and 10 and ends with harvesting in the period June 26-27.

Our data on duration of the growing season are similar to those obtained and described by Stamatov et al. (2017a) results in the period 2015-2016 in the experimental field of IRGR - Sadovo. When einkorn is sown in October, the genotypes included in the study have a growing season of 236 to 238 days.

Despite the relatively equal duration of the vegetation period, in the three experimental years differences were reported regarding the duration of the interphase periods. In the first interphase period - from germination to the third leaf phase, plants enter the fastest in 2019 (in 27 days), and the slowest (40 days) in 2020. The differences in the three years reach up to 13 days and are due to the different temperature conditions from the second ten days of November to the second ten days of December in the three experimental years. In this interphase period, plants accumulate the most active temperature sum (240.5°C) in the second experimental year (2019-2020), which explains the faster entry into the third leaf phase (Table 3).

Seven to nine days is the difference in the three years and in relation to the next interphase period - third leaf - tillering. However, crops enter the tillering phase in one calendar term, regardless of how the previous phases went. The required number of days from germination to tillering is a relatively constant value - from 43 to 47 days, and for this purpose a temperature sum of about 187°C, as accumulated in the cooler autumn of 2018 is sufficient.

In the following phases, the plants enter at the same time in the three years with a difference of a maximum of 4 calendar days until the beginning of stem elongation and with a difference of one day each in heading, milky, waxy and full maturity.

Table 2. Duration of interphase periods in *Triticum monococcum* L., number of days, 2018-2021

Interphase period	Duration of vegetation period, number of days			
	2018-2019	2019-2020	2020-2021	Average
Germination - Third leaf	36	27	40	34
Third leaf - Tillering	9	16	7	11
Tillering - Stem elongation	123	120	120	121
Stem elongation - Heading	21	24	24	21
Heading - Milk maturity	23	24	23	23
Milk maturity - Wax maturity	11	9	11	10
Wax maturity - Full maturity	7	10	8	8
Vegetation period	230	230	233	231

The average accumulated temperature during the three years is 1512°C, with the minimum sufficient effective temperature amount recorded in 2020/2021 - 1431.1°C. In combination with the largest amounts of precipitation in the March-June period, the agro-meteorological conditions in the second experimental year (2020/2021) are the main prerequisite for the highest yield.

Table 3. Sum of active temperatures by interphase periods, for *Triticum monococcum* L., °C, 2018-2021

Interphase period	Duration of vegetation period, number of days			
	2018-2019	2019-2020	2020-2021	Average
Germination - Third leaf	160.6	240.5	227.5	209.5
Third leaf - Tillering	26.2	88.7	34.4	49.8
Tillering - Stem elongation	389.4	354	260.1	334.5
Stem elongation - Heading	242.2	270.1	264.9	259.1
Heading - Milk maturity	347.1	319.4	316.1	327.5
Milk maturity - Wax maturity	216.4	138.6	165	173.3
Wax maturity - Full maturity	137.8	174.5	163.1	158.5
Vegetation period	1519.7	1585.8	1431.1	1512.2

No differences were found in the phenological phases as a result of the different fertilization variants.

Productive tillering. In the first year, 2.9 to 3.9 productive tillers develop per plant, on average from the repetitions for the different fertilization variants. In the second and third year, their number is significantly higher - from 4.3 to 4.5, and from 4.4 to 4.9 pieces, respectively.

Fertilization has different effects in different years. In the first year, the variants without fertilization and those fertilized with the soil fertilizer Italpolina have the same average values. In the second year, the control variants (without fertilization) have a higher productive tillering than those fertilized with soil fertilizer and leaf fertilizer, and in the third year, fertilization with soil fertilizer has been proven to increase the productive tillering. In all three years, Naturamin WSP did not lead to an increase in productive tillering (Table 4).

Table 4. Influence of fertilization on number of productive tillerss/plant, at the end of the growing season in *Triticum monococcum* L., by years

Variant (Fertilization)	2018/2019	2019/2020	2020/2021
Without fertilization (control)	2.9 a*	4.5 a	4.6 ab
Italpolina	2.9 a	4.4 a	4.9 a
Naturamin WSP	3.9 a	4.3 a	4.4 b

*Means followed by the same letter are not statistically different (P < 0.05) by Duncan's multiple range test

The summary statistical analysis for the three-year experimental period shows proven differences in the influence of year on productive tillering (Table 5).

Table 5. Influence of the year and fertilization on the number of productive tillers at the end of the growing season in *Triticum monococcum* L., 2018-2021

Influence of factor Year			Influence of factor Ferilization		
Variant	Number of tillers/ plant	%	Variant	Number of tillers/ plant	%
2018-2019	2.9 b*	100.0	Without fertilization (control)	4.0 a	100.0
2019-2020	4.4 a	151.7	Italpolina	4.1 a	102.5
2020-2021	4.6 a	158.6	Naturamin WSP	3.9 a	97.5

*Means followed by the same letter are not statistically different (P < 0.05) by Duncan's multiple range test

The second and third experimental years provide proven better conditions for tillering than the first year. In the third year, the plants reached a maximum of 4.6 productive tillers per plant, which is 58.6% more than the first year. Productive tillering increases as a result of soil fertilization with Italpolina, while the foliar Naturamin WSP does not affect this indicator.

Dynamics of growth in height. Plant height is an important parameter monitored by specialists and farmers, given the fact that excessive growth can lead to lodging, which negatively affects both harvesting and final yield. On the other hand, it is an indicator of active growth processes and biomass accumulation, which correlate with yield. The dynamics of growth in height was followed by measuring in three phases - tillering, stem elongation end heading (Figure 4).

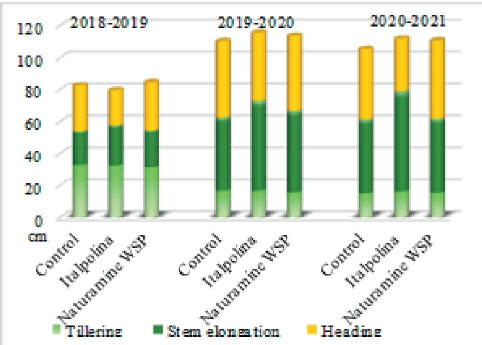


Figure 4. Dynamics of growth in height of *Tr. monococcum* L. depending on fertilization, by phenological phases and years, cm

It is clear from Figure 4 that the second and third growing years provide more favorable conditions for reaching maximum plant heights. Vegetative growth is directly dependent on rainfall, which in these two years is better distributed.

When studying the influence of the tested fertilizers on the height (Table 6), the stronger influence of the soil fertilizer is established, which is mainly manifested in the second and third year. Already during the stem elongation phase, differences of 9.7 cm in the second and 17.6 cm in the third year are reported, which, until heading, decrease to 5.3 cm and 6.4 cm, respectively.

The influence of the foliar fertilizer Naturamin is weaker, only in the stem elongation phase and only in the second year. By the heading phase, some influence was reported in all three years. The differences with the control reached 2.0 cm in the first year, 4.2 cm in the second and 5.3 cm in the third year. It can be summarized that the influence of fertilization is more significant in the more moisture-provided years 2019/2020 and 2020/2021. The summary statistical treatment for the three-year period shows the strong proven influence of the year. Italpolina leads to an increase in height by 2.9 cm, and Naturamin WSP - by 3.5 cm.

Table 6. Influence of the year and fertilization on plant height at the end of the growing season for *Triticum monococcum* L., cm, 2018-2021

Influence of factor Year			Influence of factor Ferilization		
Variant	Height, cm	%	Variant	Height, cm	%
2018-2019	82.2 b	100.0	Without fertilization (control)	99.5 a	100.0
2019-2020	113.2 a	137.7	Italpolina	102.4 a	102.9
2020-2021	109.4 a	133.1	Naturamin WSP	103.0 a	103.5

*Means followed by the same letter are not statistically different ($P < 0.05$) by Duncan's multiple range test

Zorovski et al. (2018) also conducted similar studies in Bulgaria, comparing *T. monococcum* L. with other ancient cereal species. In the period 2014-2018, on average, einkorn plants reached heights of 79.90 to 97.38 cm, which corresponds with our results.

Einkorn was also included in studies by Kirchev and Semkova (2016). They establish the influence of nitrogen fertilization on this indicator. At a nitrogen fertilizer rate of 40 kg/ha, the plants reach a height of 103.4 cm, and at 80 kg/ha - 106.2 cm.

With our results, we also confirm the influence of fertilization as a factor that can influence the growth and development of *Triticum monococcum* L.

Productive stems/m². The dynamics of stem formation gives us information about what productivity can be expected from a crop that started its vegetation with a certain number of plants and developed a certain number of tillers of each plant - non-productive and productive. The reduction of the number of plants, as well as the reduction of the tillers of each individual plant, is a natural biological process, also known as self-regulation in the crop.

Einkorn reacts in a specific way (Table 7). Even in the less favorable 2018/2019 year, it formed a relatively stable number of productive stems - from 799 pieces to 861 pieces per m^2, most likely due to the highly adaptive reactions of the species. Fertilization with soil fertilizer has a positive effect on this indicator, while foliar fertilizer is of lesser importance, and only in the second and third year.

Table 7. Influence of fertilization on the productive stem in *Triticum monococcum* L., by years, number per m^2

Variant Fertilization	2018/2019	2019/2020	2020/2021
Without fertilization (control)	799 a	496 a	963 a
Italpolina	861 a	1050 a	1020 a
Naturamin WSP	796 a	1030 a	1004 a

*Means followed by the same letter are not statistically different ($P < 0.05$) by Duncan's multiple range test

The summary data for the three-year period (Table 8) confirms the strong influence of the year. Both types of fertilization have a positive effect, but it is stronger with soil fertilizer.

Table 8. Influence of the year and fertilization on the productive stems of *Triticum monococcum* L., average for the period 2018-2021, number per m^2

Influence of factor Year			Influence of factor Ferilization		
Variant	Produc-tive stems, number/m^2	%	Variant	Produc-tive stems, number/m^2	%
2018-2019	819 b*	100.0	Without fertilization (control)	903 a	100.0
2019-2020	1009 a	123.2	Italpolina	977 a	108.2
2020-2021	996 a	121.6	Naturamin WSP	943 a	104.4

*Means followed by the same letter are not statistically different ($P < 0.05$) by Duncan's multiple range test

The correlation coefficients presented in Table 9 support our conclusions about the significance of the studied indicators. The productive tillering of the individual plants, as well as the total number of formed productive stems per m^2, is in a proven positive correlation with the yield.

Table 9. Correlation dependencies between the yield and some productivity parameters of *Tritium monococcum* L. (Correlation coefficient - R; Determination coefficient - D)

Productivity parameters	Yield kg/da	Productive tillering, number of tillers/plant		Productive stems, number/m^2	
		R	D	R	D
Yield kg/da	1.000	0.422**	18	0.371**	14
Productive tillering, Number of tillers /plant		1.000		0.419**	18
Productive stems, Number/ m^2				1.000	

**The correlation is significant at the level of $P \leq 0.01$;

CONCLUSIONS

The vegetation period of *Triticum monococcum* L. is available in 230 to 233 days (in case of autumn sowing) under the conditions of Bulgaria. The duration of the first interphase periods (from emergence to third leaf and from third leaf to tillering) strongly depends on agrometeorological conditions. In the course of their vegetation, plants accumulate an average of 1512.2°C. Productive tillering strongly depends on the conditions of the year, and less on fertilization. The influence of the soil fertilizer Italpolina is more significant. Growth in height is more intense in the more favorable second and third experimental years. Fertilization with Italpolina and Naturamin affects plant height, increasing it by 2.9 to 3.5%. Both fertilizers have a positive effect on the formed productive stem/m^2 at the end of the year. Productive tillering/plant and productive stems/m^2 have been shown to be positively correlated with yield.

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ASSESSMENT OF PHENOTYPIC DIVERSITY IN SOME WILD THYME POPULATIONS FROM BANAT AREA (WESTERN ROMANIA)

Rodica BEICU, Sorina POPESCU, Florin IMBREA, Giancarla VELICEVICI, Alina NEACȘU, Georgeta POP, Liana BUTTA, Ilinca IMBREA

University of Life Sciences "King Mihai I" from Timișoara,
119 Aradului Street, Timișoara, Romania

Corresponding author email: sorinapopescu@usvt.ro

Abstract

The paper aimed to analyse the morphological characters for eight spontaneous populations of thyme, including an endemic population (Thymus comosus), collected from different regions of the Banat area. Both interpopulation and intrapopulation variability determinations were pursued, and results were correlated with statistical interpretations. Morphological analyses were carried out, including the shape of the leaves, as this morphological character is decisive in the taxonomy of the genus Thymus. Based on morphological similarity, the analysed populations were hierarchically classified, by the cluster mean method, in two main clusters. In the first cluster, six of the eight analysed populations were grouped, respectively those belonging to the species Th. praecox (with the two subspecies janke and polytrychus) and Th. dacicus. In the second cluster we find the endemic population of Th. comosus and the population of Th. pulegioides, which are 97.51% morphologically similar, but different from the populations found in the first cluster. The obtained data allowed the quantification of the associative relationships between the morphological parameters studied.

Key words*: morphological traits, wild thyme, Thymus comosus, endemic.*

INTRODUCTION

The *Lamiaceae* (*Labiatae*) family is a family of angiosperm plants of real economic value, intensively studied from a phytotechnical, chemical and pharmaceutical aspect (Cocan et al., 2018; Guriță et al., 2019; Karpinski, 2020). The family includes over 200 genera, the *Thymus* genus being one of the numerically well-represented genera (over 200 species), used since ancient times (Morales, 1997; Morales in Stahl-Biskup & Saez, 2002). It is recognized as a genus that poses taxonomic problems, due to the variability of the species (Ložiene, 2006; Sostarić et al., 2012; Beicu et al., 2021), but of interest for the purpose of improving and identifying new local populations of interest, for the purpose of introduction into culture (Rus et al., 2016), direct harvesting, in the case of widespread species, as well as finding measures of conservation for endemic species.

The variability of the morphological traits of the analyzed populations increases the degree of selection of the desired traits for reproduction, allowing the breeder to choose the optimal variant to produce the desired hybrid (Razaei et al., 2016; Nurzynska-Wierdak et al., 2022).

We have compared in the study, common widespread, frequently used and studied populations, such as *Th. pulegioides*, but also some populations with a reduced distribution area in rocky habitats, endemic or rare and less studied. Among these, *Th. comosus* is a Carpathian endemism (mentioned only in Romania as a distribution) and *Th. dacicus* has a small distribution area only in Romania and the former Yugoslavia (Jalas, 1972; Sârbu et al., 2013; Pavel et al., 2010). The two species of *Th. praecox* are adapted to rocky areas, skeletal soils, with morpho-genetic characters of interest to breeders. All these species are also important from a phytotherapeutic point of view (Stahl-Biskup, 1991; Ložiene et al., 2007; Petrović, 2017; Alfonso et al., 2018; Vaičulite et al., 2021; Babotă et al., 2022; Babotă et al., 2023).

MATERIALS AND METHODS

The analyzed material was harvested during the vegetation period of the plants, at full bloom, in 2019, from several western areas of Banat, located at different altitude. Eight populations were analyzed, with individuals randomly selected within each population, at least 30

individuals. The phenological determinations (plant height, flower (inflorescence) length, leaf length, leaf width, leaf shape) were carried out in the laboratory. The accurate determination of the species was carried out based on specialized determinatory (Guşuleac, 1961; Sârbu et al., 2013). After identification, specimen samples for each population were stored in the herbarium of the Biology Department of the Banat's University of Life "King Michael Ist" from Timișoara.
The data obtained as a result of the biometric measurements on the morphological traits, were statistically processed, determining the estimated values of the average standard deviation and the coefficient of variability (Ciulcă, 2006).

To determine the significance of the differences between the genotypes studied, the experimental data obtained were processed by analysis of variance and the t-test (Mantel, 1967). The UPGMA method was used for the representation of the dendrogram (Yan et al., 2000).

RESULTS AND DISCUSSIONS

The analysed populations, as well as their location, are shown in Table 1.
Based on the results of the variance analysis regarding the height of the thyme populations, (Table 2), the existence of real and strongly statistically ensured differences between the studied populations in terms of plant height can be found.

Table 1. Thyme populations studied and their location

Code	Population	Location	Voucher specimen	GPS coordinates (degree, minutes, secondes)		
				Altitude (m)	Latitude	Longitude
Th1	*Th. praecox* ssp. *janke*	Domogled	VSNH.BUASTM: 1904	426	44. 884950	22.428793
Th2	*Th. dacicus*	Coronini	VSNH.BUASTM: 1910	110	44.665889	21.694774
Th3	*Th. dacicus*	Lescovita	VSNH.BUASTM: 1912	117	44.874057	21.539302
Th4	*Th. comosus*	Dobraia	VSNH.BUASTM: 1913	932	44.998398	22.480722
Th5	*Th. praecox* ssp. *polytrichus*	Gozna Peak	VSNH.BUASTM: 1918	1411	45.189375	22.073795
Th6	*Th. praecox* ssp. *polytrichus*	Semenic Peak	VSNH.BUASTM: 1919	1408	45.188707	22.076211
Th7	*Th. pulegioides ssp. pulegioides*	Semenic	VSNH.BUASTM: 1925	1004	45.224281	22.073924
Th8	*Th. praecox* ssp. *janke*	Coronini	VSNH.BUASTM: 1926	94	44.664652	21.699375

Table 2. Variance analysis for plant height and flowers length of *Thymus* populations

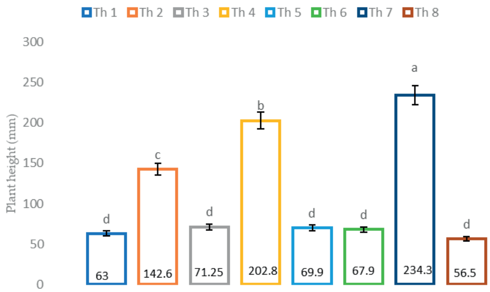
Variation source	GL	Plant height			Flower length		
	GL	SP	S^2	F test	SP	S^2	F test
Total	79	375401			25476.80		
Population	8	349786	49969	140,45**	18481.07	2640.15	27.17**
Error	72	25615	356		6995.72	97.16	

GL - degrees of freedom; SP - sum of squares; S^2 - weighted sum of squares;
Significance for F-test: ns $p > 0.05$; *$p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

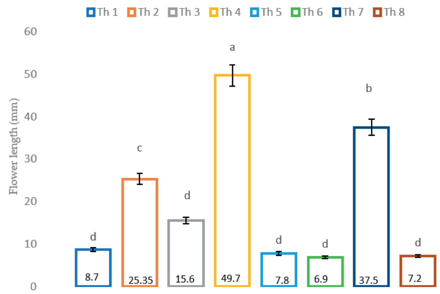
Considering the mutual comparisons between the populations, a significantly higher height of the plants can be found in the *Th. pulegioides* (Th7), by more than 31.5 mm, compared to the rest of the populations. The endemic population of *Th. comosus* (Th4), recorded significantly higher values of this character, compared to the other six populations. The highest inter-individual homogeneity of this character (Figure 1a) was recorded in the *Th. pulegioides*, followed by the *Th. praecox* ssp. *polytrichus* (Th5).
The analysis of variance components (Table 2) shows that there are distinctly significant real differences between the populations studied in terms of the inflorescences length in the conditions of the existence of a very high variability at the intra-population level, which, however, does not significantly influence the hierarchy of the populations.
The values of this character generally recorded a high variability and amplitude of variation, between 6.9 mm at the *Th. praecox* ssp. *polytrichus* (Th6) and 49.70 mm at the endemic population (Th4). Based on the multiple comparisons, it can be observed that the *Th. comosus* population, presented values of this character significantly higher, by more than 12 mm, compared to the other populations,

followed by the *Th. pulegioides*. Both populations of *Th. praecox* ssp. *janke* (Th1, Th8) and *Th. praecox* ssp. *polytrichus* (Th5, Th6), had values below 9 mm, correlated with their smaller waist.

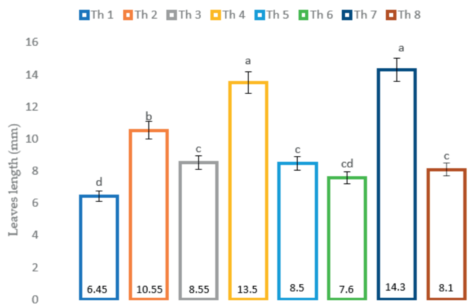
The lowest intra-population variability and amplitude was determined in the populations *Th. praecox* ssp. *polytrichus* (Th5, Th6) and *Th. praecox* ssp. *janke* (Th8), whose distributions show a certain degree of symmetry (Figure 1b).



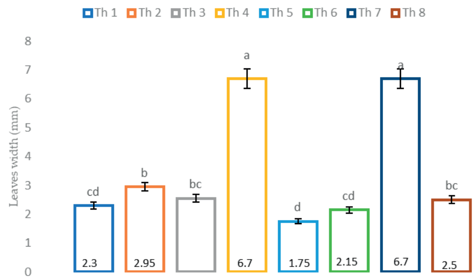
a. Variabilility of plant height (mm)



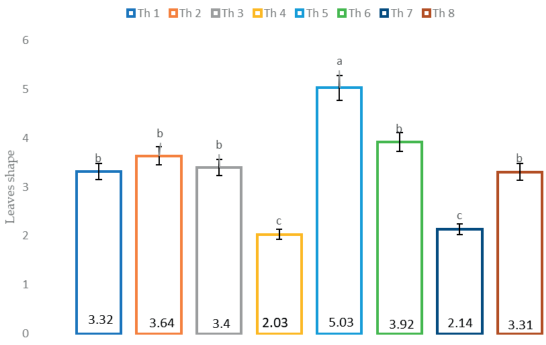
b. Variability of flower length (mm)



c. Variability of leaves length (mm)



d. Variability of leaves width (mm)



e. Variability of leaves shape index

Figure 1. Phenotypic diversity based on morphological traits

The results are expressed as the average value of three determinations ± the standard deviation (SD) indicated by the error bars. According to t-test, the different lower case letters (a - f) represent the significant differences (p < 0.05) between samples obtained by the same shredding method

The analysis of the variance for the length, width and shape of the leaves, attests that there are real and distinctly significant differences between the studied populations, from the point of view of this character, given the existence of moderate variability at the intra-population level (Table 3).

Inter-individual variability for leaf length was higher in *Th. praecox* ssp. *polytrichus* (Th6), while in *Th. dacicus* (Th3), *Th. pulegioides* (Th7) and *Th. praecox* ssp. *janke* (Th8) populations, leaf uniformity was considerably higher (Figure 1c). Based on the multiple comparisons, it can be observed that the *Th.*

pulegioides (Th7) and *Th. comosus* (Th4) populations, presented a significantly higher leaf length, than the other populations, with more than 2.95 mm.

Table 3. Variance analysis for length, width and shape index of leaves for *Thymus* populations

Variation source	Leaves lenght				Leaves width			Shape index		
	GL	SP	S^2	F test	SP	S^2	F	SP	S^2	F test
Total	79	741.75	80.88		325.80			135.70		
Population	8	566.17	2.44	33.17**	289.90	41.41	83.06**	64.44	9.21	9.30**
Error	72	175.58			35.90	0.50		71.26	0.99	

GL - degrees of freedom, SP- sum of squares, S^2- weighted sum of squares, Significance for F-test: ns $p>0.05$; *$p\leq0.05$; ** $p\leq0.01$; *** $p\leq0.001$

The width of the leaves in the eight populations, recorded values between 1.75 mm at *Th. praecox* ssp. *polytrichus* (Th5) and 6.70 mm at *Th. comosus* (Th4) and *Th. pulegioides* (Th7), respectively, with an amplitude of variation of 4.95 mm and a high inter-population variability, associated with moderate heterogeneity at the level intrapopulation (Table 3). A significantly higher width of the leaves was also found in *Th. comosus* (Th4) and *Th. pulegioides* (Th7) populations, by more than 3.75 mm compared to the rest of the populations. The other populations presented values below 3 mm and generally not statistically assured (Figure 1d).

Regarding the leaf shape index, the inter-individual variability was higher in the *Th. praecox* ssp. *janke* (Th1) and *Th. praecox* ssp. *polytrichus* (Th6) populations, respectively lower in the *Th. pulegioides* (Th7), and *Th. dacicus* (Th3) populations (Table 3). Considering the mutual comparisons between the populations, it is observed that the population *Th. praecox* ssp. *polytrichus* (Th5), presented the highest degree of leaf elongation associated with significant differences, compared to the shape of the leaves in the rest of the analysed populations. Populations *Th. praecox* ssp. *janke* (Th1), *Th. dacicus* (Th2, Th3), *Th. praecox* ssp. *polytrichus* (Th6), *Th. praecox* ssp. *janke* (Th8), made up a class of significance, presenting a more elongated shape of the leaves compared to populations *Th. comosus* (Th4) and *Th. pulegioides* (Th7) (Figure 1e).

Regarding the data presented in the similarity matrix, the highest phenotypic similarity is manifested between the two populations of *Th. praecox* ssp. *janke* (Th1, Th8), analysed from different locations - 99.03%; *Th. praecox* ssp. *janke* (Th8) and *Th. dacicus* (Th3) - 99.01%; *Th. praecox* ssp. *janke* (Th8) and *Th. praecox* ssp. *polytrichus* (Th6) - 98.91%; *Th. praecox* ssp. *polytrichus* (Th6) and *Th. praecox* ssp. *janke* (Th1) -98.70%; *Th. praecox* ssp. *polytrichus* (Th6) and *Th. dacicus* (Th3) - 98.14% (Table 4).

Table 4. The similarities coefficients between *Thymus* populations for analysed morphological traits

Population	1	2	3	4	5	6	7	8
Th. praecox ssp. *janke* (Th1)	1							
Th. dacicus (Th2)	0.8694	1						
Th. dacicus (Th3)	0.9794	0.9418	1					
Th. comosus (Th4)	0.3365	0.7117	0.5018	1				
Th.praecox ssp. *polytrichus* (Th5)	0.9185	0.8646	0.9291	0.2154	1			
*Th.*praecox ssp. *polytrichus* (Th6)	0.9870	0.8923	0.9814	0.3235	0.9686	1		
Th. pulegioides ssp. *pulegioides* (Th7)	0.3348	0.7203	0.4963	0.9751	0.2379	0.3375	1	
Th. praecox ssp. *janke* (Th8)	0.9903	0.8936	0.9901	0.3923	0.9280	0.9891	0.4006	1

The dendrogram (Figure 2), based on morphological similarity, groups the analysed populations into two main clusters. The first cluster has a complex structure, being made up of six populations, between which there is an average similarity of approximately 90%. Only two thyme populations were grouped in the second cluster *Th. comosus* (Th4) and *Th. pulegioides* (Th7), which are morphologically similar to a degree of 97.51%, at the same time differing by approximately 68% compared to the populations of the first cluster.

Regarding the variance analysis for the analysed morphological characters (Table 5), it is found that the width of the leaves recorded higher and significant values of the variance, thus contributing to a greater extent (23.50%) to the diversity between the populations. The shape of the leaves had a smaller influence (16.05%) on the morphological differentiation of the populations, while the rest of the characters had close contributions (19.79-20.44%). Within the clusters, morphological diversity was generated to a higher extent (38.53%) by leaf shape.

Based on the variance analysis related to the hierarchical classification of the populations (Table 6), it is observed that *Th. praecox* ssp. *polytrichus* (Th5, Th6), generate the greatest differences between the studied morphological characters, having high contributions to the total variability. The *Th. praecox* ssp. *polytrichus* (Th5) population, registers the highest effect (47.34%), on the diversity at the level of the first cluster, while the Th. dacicus (Th2, Th3) populations, influence the diversity to an extremely low extent (0.33%). In the case of the second cluster, the *Th. pulegioides* (Th7) population, has a higher contribution (56.34%) than the *Th. comosus* (Th4) population (43.66%).

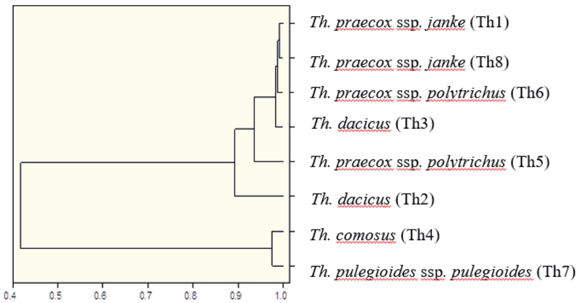


Figure 2. Cluster analysis based on morphological traits

Table 5. Variance analysis for the morphological traits for *Thymus* populations

Character	Between		Within		F test
	SP	GL	SP	GL	
Plant height	5.886	1	1.114	6	31.69**
Flower length	5.699	1	1.299	6	26.31**
Leaves length	5.850	1	1.170	6	30.00**
Leaves width	6.768	1	0.198	6	205.26**
Index shape	4.621	1	2.370	6	11.70**

GL - degrees of freedom, SP- sum of squares, Significance for F-test: ns $p>0.05$; *$p\leq0.05$; ** $p\leq0.01$; *** $p\leq0.001$

Table 6. Variance analysis for *Thymus* populations based on morphological traits

Population	Between		Within		F test
	SP	GL	SP	GL	
Th. praecox ssp. *janke* (Th1)	4.864	1	0.882	3	16.54*
Th. dacicus (Th2)	5.186	1	0.186	3	83.76**
Th. dacicus (Th3)	5.826	1	0.189	3	92.30**
Th. comosus (Th4)	0.159	1	3.569	3	0.13
Th. praecox ssp. *polytrichus* (Th5)	13.392	1	2.663	3	15.09*
*Th.*praecox ssp. *polytrichus* (Th6)	7.905	1	1.221	3	19.43*
Th. pulegioides ssp. *pulegioides* (Th7)	0.528	1	4.593	3	0.34
Th. praecox ssp. *janke* (Th8)	6.500	1	0.484	3	40.26**

GL - degrees of freedom, SP - sum of squares, Significance for F-test: ns $p > 0.05$; *$p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$

The multivariate analysis (Figure 3) based on the first two main components expresses 99.98% of the variability of the five morphological characters in the analysed populations. In the case of the populations of second cluster, the plants had a higher height, associated with a larger size of the inflorescences and leaves.

In Table 7, it is observed that 90.63% (Th1) and respectively 83.34% (Th8), of the variability of the length of the inflorescence in the populations of *Th. praecox* ssp. *janke*, can be explained as the result of the influence of the four characters. Plant height, for *Th. praecox ssp. janke* from Domogled (Th1), has a major (82.82 %) and distinctly significant contribution to the realization of the size of the inflorescence, followed by the width of the leaves which influences to an extent of approximately 7.3% the variability of this character, while the length and leaf shape had a very low influence of about 0.3%. The variability of inflorescence length in this population was caused to a degree of 9.4% by environmental conditions or other factors. For *Th. praecox* ssp. *janke* from Coronini (Th8), the length of the leaves has a distinctly significant major influence of approximately 64.71% on the realization of that character, being followed by the shape of the leaves which influences the size of the inflorescence to a degree of 13.48%.

Based on the data presented in Table 8, it is observed that the length of the inflorescence for *Th. praecox* ssp. *janke* (Th1), shows positive and strongly statistically assured correlations with respect to plant height and leaf width, except for leaf length where the correlation coefficient is positive but does not reach the level of significance. The shape of the leaf shows a negative correlation, as such the elongation of the shape of the leaves is associated with a smaller size of the inflorescences. Plant height shows a significantly positive correlation with leaf width and, respectively, a very high negative correlation with leaf shape. Thus, in the case of this population, the short stature of the plants was associated with the elongated shape of the leaves.

Analysis of the correlation coefficients between the five morphological characters studied in the population of *Th. praecox* ssp. *janke* (Th8) (Table 8), shows the existence of low and statistically uncertain correlations between the length of the inflorescence and the other characters, in contradiction with the situation presented previously. The differences between the character structure of the two populations are due to the different ecological conditions in Domogled (Th1) and Coronini (Th8).

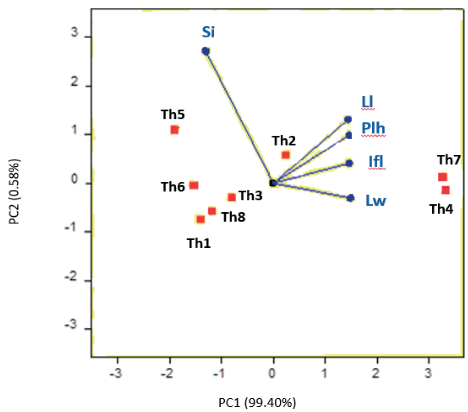


Figure 3. Biplot diagram for morphological traits

Plh-plant height; Ifl-Inflorescence length; Ll-leaves length; Lw-leaves width; Si-Shape index

Table 7. Variance components of multiple regression between flowers length and other morphological traits for *Th. praecox* ssp. *janke*

	***Th. praecox* ssp. *janke* (Th1)**				***Th. praecox* ssp. *janke* (Th2)**			
Source of variability	**SP**	**GL**	**S^2**	**F test**	**SP**	**GL**	**S^2**	**F test**
Regression	119.40 (90.63 %)	4	29.85	36.26**	8.28 (83.34 %)	4	2.07	18.71**
Plant height (x_1)	109.12 (82.82 %)	1	109.12	132.53**	0.03 (0.28 %)	1	0.03	0.25
Leaves length (x_2)	0.30 (0.22 %)	1	0.30	0.36	6.43 (64.71 %)	1	6.43	58.12**
Leaves width (x_3)	9.64 (7.32 %)	1	9.64	11.71**	0.48 (4.86 %)	1	0.48	4.37
Index shape (x_4)	0.35 (0.27 %)	1	0.35	0.43	1.34 (13.48 %)	1	1.34	12.11**
Other sources	12.35 (9.37 %)	15	0.82		1.66 (16.70 %)	15	0.11	
Total	131.75	19			9.94	19		

Th1: $y = -1.2 + 0.29x_1 - 0.14x_2 + 0.82x_3 + 0.13x_4$; $R^2 = 0.9063$; DW = 2.2; SDE = 2.22 mm;
Th8: $y = -6.22 + 0.09x_1 - 5.97x_2 + 11.92x_3 + 8.07x_4$; $R^2 = 0.8334$; DW = 2.12; SDE = 0.81 mm

Table 8. Correlation coefficients values between studied morphological traits for *Th. praecox* ssp. *janke*

Character	*Th. praecox* ssp. *janke* (Th1)					*Th. praecox* ssp. *janke* (Th2)				
	Plant height	Leaves length	Leaves width	Index shape	Flower length	Plant height	Leaves length	Leaves width	Index shape	Flower length
Plant height	**1.000**	0.337	0.892***	-0.820^{000}	0.870***	**1.000**	0.783***	0.634**	-0.384	0.049
Leaves length		**1.000**	0.535*	-0.442	0.336		**1.000**	0.799***	-0.447	-0.424
Leaves width			**1.000**	-0.923^{000}	0.885***			**1.000**	-0.891^{000}	-0.208
Index shape				**1.000**	-0.805^{000}				**1.000**	0.012
Flower length					**1.000**					**1.000**

Th1: $r_{5\%} = 0.444$; $r_{1\%} = 0.561$; $r_{0.1\%} = 0.679$; Th8: $r_{5\%} = 0.444$; $r_{1\%} = 0.561$; $r_{0.1\%} = 0.679$

According to the data in Table 9, it is observed that the direct effects of plant height, length and shape of leaves on the correlation with the length of inflorescences, for *Th. praecox* ssp. *janke* from Domogled (Th1), are reduced (10.98-24.75%), so the indirect effects of other characters must also be taken into account. The relationship between the length of the inflorescence and the width of the leaf is mostly due to the direct effect of the respective character (64.14%), against the background of reduced indirect effects from the other characters. Also, the width of the leaves has major indirect effects of 59.35-63.57% on the connection of the other characters with the length of the inflorescence.In the case of *Th. praecox* ssp. *janke* from Coronini (Th8), the analysis of the path coefficients (Table 9) confirms that the relationship between the length of the inflorescence and the width of the leaves shows the highest stability (42.80%), while the relationship between the length of the inflorescence and the height of the plants has the lowest stability (13.48%). The width of the leaves also showed a high indirect influence in this population (36.81-45.40%) on the length of the inflorescence through the other morphological characters.

Table 9. Path coefficients values for flowers length for *Th. praecox* ssp. *janke* and *Th dacicus*

Correlative links	Th1		Th8		Th2		Th3	
	Path coefficients							
Flower length - Plant height	Value	%	Value	%	Value	%	Value	%
The direct effect of plant height	0.293	24.75	1.149	13.48	-0.734	24.70	0.310	9.09
The indirect. through the leaves length	-0.049	4.12	-3.038	35.63	-0.540	18.16	-0.460	13.50
through the leaves width	0.734	62.03	3.139	36.81	1.354	45.56	1.721	50.50
through the index shape	-0.108	9.10	-1.200	14.08	-0.344	11.59	-0.918	26.92
Total correlation	0.870	100.00	0.049	100.00	-0.264	100.00	0.653	100.00
Flower length - Leaves length	Value	%	Value	%	Value	%	Value	%
The direct effect of leaves length	-0.145	19.50	-3.879	38.29	-1.192	38.08	-1.166	44.11
The indirect. through the plant height	0.099	13.32	0.900	8.88	-0.332	10.61	0.122	4.63
through the leaves width	0.440	59.35	3.953	39.02	1.238	39.53	1.273	48.18
through the index shape	-0.058	7.82	-1.398	13.80	0.369	11.77	0.082	3.09
Total correlation	0.336	100.00	-0.424	100.00	0.082	100.00	0.312	100.00
Index shape - Leaves width	Value	%	Value	%	Value	%	Value	%
The direct effect of width leaves	0.823	64.14	4.949	42.80	2.690	52.31	2.501	53.97
The indirect. through plant height	0.261	20.37	0.729	6.30	-0.370	7.19	0.213	4.60
through leaves length	-0.077	6.03	-3.098	26.79	-0.549	10.67	-0.594	12.81
through index shape	-0.121	9.45	-2.788	24.11	-1.534	29.83	-1.326	28.62
Total correlation	0.885	100.00	-0.208	100.00	0.238	100.00	0.794	100.00
Flower length - Index shape	Value	%	Value	%	Value	%	Value	%
The direct effect of index shape	0.131	10.98	3.130	32.22	1.981	44.90	1.607	41.14
The indirect. prin plant height	-0.240	20.10	-0.441	4.54	0.128	2.89	-0.177	4.53
through leaves length	0.064	5.35	1.733	17.84	-0.222	5.03	-0.059	1.51
through leaves width	-0.760	63.57	-4.409	45.40	-2.082	47.18	-2.063	52.82
Total correlation	-0.805	100.00	0.012	100.00	-0.195	100.00	-0.692	100.00

The analysis of the variance of the multiple regression, regarding the influence of different morphological characters on the size of the inflorescence, in the two populations of *Th. dacicus,* indicates that, the variability of the length of the inflorescence is due to the influence of the four characters, to a significant extent of 51.89% for the species from Coronini (Th2) and 83.27% for the one from Lescovița (Th3) (Table 10). The length of the inflorescences in the population of *Th. dacicus* from Lescovita showed a higher stability compared to the population from Coronini.

Table 10. Variance components of multiple regression between flowers length and other morphological traits for *Th. dacicus* (Th2, Th3)

	***Th. dacicus* (Th2)**				***Th. dacicus* (Th3)**			
Source of variability	**SP**	**GL**	**S^2**	**F test**	**SP**	**GL**	**S^2**	**F test**
Regression	598.20 (51.86 %)	4	149.55	4.04*	69.85 (83.27 %)	4	17.46	18.68**
Plant height (x_1)	119.20 (10.33 %)	1	119.20	3.22	41.81 (49.84 %)	1	41.81	44.73**
Leaves length (x_2)	87.10 (7.55 %)	1	87.10	2.35	0.33 (0.40 %)	1	0.33	0.36
Leaves width (x_3)	245.40 (21.27 %)	1	245.40	6.63*	23.15 (27.59 %)	1	23.15	24.76**
Index shape (x_4)	146.50 (12.70 %)	1	146.50	3.96	4.57 (5.44 %)	1	4.57	4.89*
Other sources	555.45 (48.15 %)	15	37.03		14.02 (16.71 %)	15	0.93	
Total	1153.55	19			83.88	19		

Th2: $y = y = -162 -0.53x_1 -14.7x_2 +84.7x_3 +46.4x_4$; $R^2 = 0.5186$; DW = 2.03; SDE = 4.9 mm;
Th3: $y = y = -40.8 +0.05x_1 -5.31x_2 +22.4x_3 +12.1x_4$; $R^2 = 0.8327$; DW = 1.75; SDE = 2.37 mm

The study of the correlations indicates, the existence of very low and statistically uncertain links between the length of the inflorescences and the rest of the morphological characters, for the population from Coronini (Th2). The closest positive and significant correlations are observed between the height of the plants and the dimensions of the leaves, while the width of the leaves shows a high negative correlation with their shape. In the case of the population from Lescovița (Th3), the length of the inflorescence shows positive and strongly statistically assured correlations with respect to the height of the plants and the width of the leaves, while the correlation coefficient with respect to the length of the leaf is positive but does not reach the level of significance (Table 11).

Table 11. Correlation coefficients values between studied morphological traits for *Th. dacicus* (Th2, Th3)

Character	***Th. dacicus* (Th2)**					***Th. dacicus* (Th3)**				
	Plant height	Leaves length	Leaves width	Index shape	Flower length	Plant height	Leaves length	Leaves width	Index shape	Flower length
Plant height	**1.000**	0.453*	0.503*	-0.174	-0.264	**1.000**	0.395	0.688***	-0.571^{00}	0.653**
Leaves length		**1.000**	0.460*	0.186	0.082		**1.000**	0.509*	0.051	0.312
Leaves width			**1.000**	-0.774^{000}	0.238			**1.000**	-0.825^{000}	0.794***
Index shape				**1.000**	-0.195				**1.000**	-0.692^{000}
Flower length					**1.000**					**1.000**

Th2: $r_{5\%} = 0.444$; $r_{1\%} = 0.561$; $r_{0.1\%} = 0.679$; Th3: $r_{5\%} = 0.444$; $r_{1\%} = 0.561$; $r_{0.1\%} = 0.679$

In the case of both populations, the relationship between the length of the inflorescence and the width of the leaf is mostly due to its direct effect (50%), against the background of smaller indirect effects from the other characters. Also, the shape of the leaf has important direct effects on the connection with the size of the inflorescence (Table 9).

The variability of the length of the inflorescence in the two populations of *Th. praecox* ssp. *polytrychus*, is due to a distinctly significant extent to the influence of the four characters included in the regression model. The shape of the leaves has an important and significant contribution to the inflorescence, while the width of the leaves, the length of the leaves and the height of the plant have smaller and insignificant influences on the size of the inflorescences. The size of the inflorescence in the population from the Semenic Peak area (Th6) was influenced to a degree of 38.12% by environmental conditions or other factors, showing a higher stability compared to the population belonging to the same species, harvested from the Gozna Peak, where the influence on some external factors was 49.33% (Table 12).

Table 12. Variance components of multiple regression between flowers length and other morphological traits for *Th. praecox* ssp. *polytrichus*

	Th. praecox ssp. *polytrichus* (Th5)				*Th. praecox* ssp. *polytrichus* (Th6)			
Source of variability	**SP**	**GL**	**S^2**	**F test**	**SP**	**GL**	**S^2**	**F test**
Regression	10.61 (50.66%)	4	2.65	3.85*	14.74 (61.89%)	4	3.69	6.09**
Plant height (x_1)	0.07 (0.33%)	1	0.07	0.10	0.26 (1.09%)	1	0.26	0.43
Leaves length (x_2)	0.35 (1.65%)	1	0.35	0.50	0.17 (0.69%)	1	0.17	0.27
Leaves width (x_3)	2.05 (9.77%)	1	2.05	2.97	0.49 (2.06%)	1	0.49	0.81
Index shape (x_4)	8.15 (38.91%)	1	8.15	11.83**	13.83 (58.05%)	1	13.83	22.84**
Other sources	10.33 (49.33%)	15	0.69		9.08 (38.12%)	15	0.61	
Total	20.94	19			23.82	19		

Th5: $y = 48.76 + 0.01x_1 + 3.39x_2 - 21.2x_3 - 6.63x_4$; $R^2 = 0.5066$; DW = 3.28; SDE = 2.49 mm
Th6: $y = 25.53 - 0.06x_1 + 1.38x_2 - 6.40x_3 - 2.90x_4$; $R^2 = 0.6189$; DW = 1.91; SDE = 1.89 mm

The analysis of the correlation coefficients between the morphological characters studied in these two populations, prove the existence of some low and statistically uncertain correlations between the length of the inflorescence and the other characters. The height of the plants showed a significantly positive correlation with the width of the leaves, respectively a significantly negative relationship with the shape of the leaves. There is an increase in the height of the plants, associated with a rounder shape of the leaves, respectively a higher value of their width. In the case of the shape of the leaves, close positive correlations are observed with respect to the length and negative with respect to the width of the leaves, for both analyzed populations (Table 13).

Table 13. Correlation coefficients values between studied morphological traits for *Th. praecox* ssp. *polytrichus*

	Th. praecox ssp. *polytrichus* (Th5)					*Th. praecox* ssp. *polytrichus* (Th6)				
Character	Plant height	Leaves length	Leaves width	Index shape	Flower length	Plant height	Leaves length	Leaves width	Index shape	Flower length
Plant height	**1.000**	0.046	0.673**	-0.451^{0}	-0.041	**1.000**	0.194	0.066	-0.052	0.089
Leaves length		**1.000**	0.169	0.640**	0.089		**1.000**	-0.140	0.667**	0.087
Leaves width			**1.000**	-0.642^{00}	0.146			**1.000**	-0.786^{000}	-0.125
Index shape				**1.000**	-0.095				**1.000**	-0.021
Flower length					**1.000**					**1.000**

Th5: $r_{5\%} = 0.444$; $r_{1\%} = 0.561$; $r_{0.1\%} = 0.679$; Th6: $r_{5\%} = 0.444$; $r_{1\%} = 0.561$; $r_{0.1\%} = 0.679$

The study of the path coefficients confirms that the link between the length of the inflorescence and the dimensions of the leaves shows close levels of stability for both populations (50%), while the link between the length of the inflorescence and the height of the plants has the lowest stability (below 1%). Only in the case of the shape of the leaves, close positive correlations are observed with respect to the length and negative with respect to the width of the leaves, so the relationships between these characters of the leaves are similar in the two populations (Table 14).
Analysis of the variance of the multiple regression regarding the influence of different morphological characters on the size of the inflorescence in the population of *Th. comosus* (Th4), shows that 78.78% of the variability of the length of the inflorescence is due to the influence of the four characters. The shape of the leaves has a major (43.85%) and distinctly significant contribution to the realization of the size of the inflorescence, followed by the size of the plants, which influences to an extent of approximately 22.03% the variability of this character. The variability of the length of the inflorescence depends to an extent of 21.22% on environmental conditions or other factors (Table 15).
The length of the inflorescence shows low correlations, mainly negative and not statistically assured compared to the other four characters. The length of the leaves shows significantly positive correlations with respect to the width and shape of the leaves, indicating that the increase in the size of the leaves is associated with an elongation of their shape. (Table 16). In this endemic population, the direct effects of plant height on the correlation with the length of inflorescences are lower (17.11%), so the connection between these two characters is mainly influenced by the indirect effects of the other

characters. The height of the plants also shows low indirect effects, so the change in the height of the plants of this population was not associated with significant variations of the other characters (Table 14).

Table 14. Path coefficients value for flowers length for *Th. praecox* ssp. *polytrichus*, *Th. comosus*, *Th. pulegioides*

	Th5		Th6		Th4		Th7	
Correlative links	**Path coefficients**							
Flower length - Plant height	**Value**	**%**	**Value**	**%**	**Value**	**%**	**Value**	**%**
The direct effect of plant height	0.039	0.83	-0.258	29.24	-0.480	17.11	0.255	9.05
The indirect. through the leaves length	0.168	3.61	0.337	38.20	1.188	42.39	-0.333	11.80
through the leaves width	-2.346	50.44	-0.139	15.72	-0.848	30.25	-0.740	26.27
through the index shape	2.099	45.12	0.149	16.84	-0.287	10.24	1.490	52.88
Total correlation	**-0.041**	**100.00**	**0.089**	**100.00**	**-0.426**	**100.00**	**0.672**	**100.00**
Flower length - Leaves length	**Value**	%	**Value**	%	**Value**	%	**Value**	%
The direct effect of leaves length	3.656	50.59	1.735	43.72	-5.490	50.64	-4.480	48.26
The indirect. through the plant height	0.002	0.02	-0.050	1.26	0.104	0.96	0.019	0.20
through the leaves width	-0.590	8.16	0.293	7.37	2.628	24.24	2.620	28.23
through the index shape	-2.979	41.22	-1.891	47.64	2.620	24.17	2.163	23.31
Total correlation	**0.089**	**100.00**	**0.087**	**100.00**	**-0.138**	**100.00**	**0.323**	**100.00**
Index shape - Leaves width	**Value**	%	**Value**	%	**Value**	%	**Value**	%
The direct effect of width leaves	-3.487	48.97	-2.093	45.70	4.738	47.94	5.108	50.48
The indirect. through plant height	0.026	0.36	-0.017	0.37	0.086	0.87	-0.037	0.37
through leaves length	0.619	8.69	-0.243	5.30	-3.045	30.81	-2.298	22.71
through index shape	2.988	41.97	2.228	48.63	-2.014	20.38	-2.676	26.44
Total correlation	**0.146**	**100.00**	**-0.125**	**100.00**	**-0.236**	**100.00**	**0.098**	**100.00**
Flower length - Index shape	**Value**	%	**Value**	%	**Value**	%	**Value**	%
The direct effect of index shape	-4.655	50.32	-2.835	50.18	4.959	50.54	4.936	50.66
The indirect. prin plant height	-0.017	0.19	0.014	0.24	0.028	0.28	0.077	0.79
through leaves length	2.340	25.29	1.157	20.47	-2.901	29.56	-1.963	20.14
through leaves width	2.238	24.20	1.645	29.11	-1.924	19.61	-2.769	28.41
Total correlation	**-0.095**	**100.00**	**-0.021**	**100.00**	**0.162**	**100.00**	**0.282**	**100.00**

Table 15. Variance components of multiple regression between flowers length and other morphological traits for *Th. comosus* and *Th. pulegioides* ssp. *pulegioides*

	***Th. comosus* (Th4)**				***Th. pulegioides* ssp. *pulegioides* (Th7)**			
Source of variability	**SP**	**GL**	**S^2**	**F test**	**SP**	**GL**	**S^2**	**F test**
Regression	631.82 (78.78 %)	4	157.96	13.93**	2455.4 (76.23 %)	4	613.85	12.03**
Plant height (x_1)	176.68 (22.03 %)	1	176.68	15.58**	1801.4 (55.93 %)	1	1801.40	35.29**
Leaves length (x_2)	54.00 (6.73 %)	1	54.00	4.76**	298.3 (9.26 %)	1	298.30	5.84*
Leaves width (x_3)	49.50 (6.17 %)	1	49.50	4.36	15.0 (0.47 %)	1	15.00	0.29
Index shape (x_4)	351.64 (43.85 %)	1	351.64	31.00**	340.7 (10.58 %)	1	340.70	6.68*
Other sources	170.14 (21.22 %)	15	11.34		765.6 (23.77 %)	15	51.04	
Total	801.96	19	157.96	13.93	3220.9	19		

Th4: $y = 248 -0.15x_1 -30x_2 +51.9x_3 +189.7x_4$; $R^2 = 0.7878$; DW = 2.41; SDE = 8.25 mm
Th7: $y = 248 -0.15x_1 -30x_2 +51.9x_3 +189.7x_4$; $R^2 = 0.7623$; DW = 1.95; SDE = 7.5 mm

Table 16. Correlation coefficients values between studied morphological traits for *Th. comosus* and *Th. pulegioides* ssp. *pulegioides*

Character	***Th. comosus* (Th4)**					***Th. pulegioides* ssp. *pulegioides* (Th7)**				
	Plant height	Leaves length	Leaves width	Index shape	Flower length	Plant height	Leaves length	Leaves width	Index shape	Flower length
Plant height	**1.000**	-0.216	-0.179	-0.058	-0.426	**1.000**	0.074	-0.145	0.302	0.672**
Leaves length		**1.000**	0.555*	0.528*	-0.138		**1.000**	0.513*	0.438	0.323
Leaves width			**1.000**	-0.406	-0.236			**1.000**	-0.542^0	0.098
Index shape				**1.000**	0.162				**1.000**	0.282
Flower length					**1.000**					**1.000**

Th4: $r_{5\%} = 0.444$; $r_{1\%} = 0.561$; $r_{0.1\%} = 0.679$; Th7: $r_{5\%} = 0.444$; $r_{1\%} = 0.561$; $r_{0.1\%} = 0.679$

Regarding the population of *Th. pulegioides* ssp. *pulegioides* (Th7), 76.23% of the variability of the length of the inflorescence, can be explained as being the result of the influence of the four characters. The height of the plants has a distinctly significant major influence (55.93%), when achieving the respective character, followed by the shape of the leaves (10.58%). Changing the width of the leaves (0.47%) had smaller and insignificant influences on the size of the inflorescences (Table 15). The analysis of the correlation coefficients shows the existence of small positive correlations and not statistically ensured between the length of the inflorescence and the other characters. The height of the plants showed a significantly positive correlation with the length of the inflorescences, thus indicating that an increase in the height of the plants of this population is associated with an increase in the size of the inflorescences against the background of small and insignificant variations in the characters of the leaves (Table 16). The study of the path coefficients presented in Table 14, proves that the relationship between the length of the inflorescence and the dimensions of the leaves shows close levels of stability (48.26-50.66%), while the link between the length of the inflorescence and the height of the plants has the lowest stability (below 10%). The smaller direct effects of the leaf sizes (around 50%) on their links with the length of the inflorescence, explain the insignificant values of the related correlation coefficients.

CONCLUSIONS

The quantitative morphological characterization based on biometric measurements, as well as the quantification of the associative relationships between the morphological parameters studied, allowed the quantification of the degree of phenotypic similarity between the analysed populations. Morphological differences are confirmed between the species of the same genera harvested from different areas, which explains the difficulty of precise taxonomic determination, at the subspecies level by classical methods.

The results of the research are extremely useful to the breeders, knowing the continuous interest in finding valuable local populations, more resistant to diseases and pests but also better adapted to the biotope conditions. Biochemical studies are recommended to recommend these populations also in terms of volatile oil quality.

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AN OVERVIEW ON BEEKEEPING AND HONEYBEE COLONY LOSSES IN ROMANIA, RESULTING FROM NATIONAL SURVEYS

Eliza CĂUIA, Nicoleta ION

Institute for Beekeeping Research and Development, Honey Bee Breeding Laboratory, Melliferous Flora and Pollination Laboratory, 42 Ficusului Blvd, 013975, Bucharest, Romania

Corresponding author email: eliza.cauia@icdapicultura.ro

Abstract

The use of questionnaire in beekeeping has been proved as an important tool of collecting valuable statistic data and answer particular questions. In the period 2014-2023 we launched different questionnaires at national level with the main purpose to evaluate the situation of honeybee colony losses (Apis mellifera) and possible causes. The main results on different questions are illustrated and analysed in the present paper, offering a general view on Romanian beekeeping, honeybee colony losses and associated causes. The preliminary data on validated questionnaires show that, the rate of honeybee colony loss registered values between 5.0% in 2015 and 17.0% in 2022/2023 winter season. Out of the total number of respondents and on the whole surveyed period, an average of 32.7% respondents registered mortalities over 10% at the apiary level, and 56.5% registered depopulations over 10%, with variations between different periods (years, months). Having an overview on the rate and incidence of colony losses in the last years and possible factors affecting honeybees can help the involved stakeholders to support the beekeeping field and adapt it to future challenges.

Key words: *beekeeping, honeybee colony losses, questionnaire, survey, Romania.*

INTRODUCTION

The honey bees (*Apis mellifera* L.) have an important contribution to the welfare and balance of natural ecosystems as well as in the human nutrition and health (Goulson et al., 2015; Cherbuliez, 2013; Gallai et al., 2009). Both, for production and pollination, the honeybee colonies have to be well developed. To capitalize the main nectar flows, the beekeepers have to implement an optimal management of honeybee population in different periods of the year, taking into account the biologic cycle and natural local conditions (Graham, 1999). To overpass the critical periods (drought, winter), the colony strength and physiologic parameters are very important, too (Somerville, 2005).
Romania's climate is a transitional temperate-continental, marked by some oceanic, continental, Scandinavian-Baltic, Mediterranean and Pontic climatic influences (https://www.meteoromania.ro).
The local honey bee (*A. m. carpatica)* (Foti et al., 1965), reconfirmed by recent studies (Oleksa et al., 2023; Chen et al., 2022; Tofilski et al., 2021; Momeni et al., 2021) is very important to be preserved for sustainable breeding and beekeeping as natural patrimony. The selection pressure, by natural (e.g. flora, temperature, diseases) and artificial conditions (e.g. beekeepers' management, flora and land use) could have a negative impact on honey bee diversity and resilience (Meixner et al., 2010; de la Rua et al., 2009).
In Romania, the beekeeping management follows specific stages correlated with the seasons, beginning with winter honeybee rearing period (August-October), continuing with the wintering period (November-February), partially overlapped by the period of replacing the wintering bee (January-April), followed by the swarming period (end April-June) which covers also the largest period of honey production (Mateescu et al., 2012). The differences in the length of active and inactive seasons depend on climatic yearly variations, altitude or latitude. Thus, the beekeeper's management should aim the maximization of biologic potential to develop or maintain the population by controlling the reproduction, space, nutrition, storages, swarming, diseases and pests, etc.
Independent or dependent of beekeeper's management, a series of population losses occur each year as seasonal losses, connected with

many causing factors. In wintering season, in Romania, the colony losses at the level of an apiary are considered acceptable under 10% (Mateescu et al., 2012), similarly with other European countries (Gray et al., 2019). In USA a threshold of up to 19.8% was recently calculated to be acceptable for different categories of apiaries (Bruckner et al., 2023; Kulhanek et al., 2017). This difference is expected because the beekeepers' structure regarding the operation size is very different in the two regions, with larger, commercial apiaries in USA, than in EU, comprising the most part of country stock. Generally, the losses could take the form of mortalities (totally lost colonies) and/or depopulations (weak colonies) with different levels of population loss (van der Zee et al., 2013). As a consequence, the population and apiary's profitability are affected proportionally with the level of population loss.

The loss rates in autumn, winter, early spring or summer are very important as they influence the number of production colonies in the next active season. To make data comparable and to guideline different stakeholders for conducting effective surveys to identify the winter colony loss rates and risk factors, a series of standardized questionnaires as well as recommendations were developed and published within COLOSS - Honey Bee Research Association - Citizen Science "Colony Losses Monitoring" Group (van der Zee et al., 2013; https://coloss.org). The standardized survey for winter colony loss monitoring was developed to measure the colony loss rates in spring based on the quantification of colonies' number at the beginning of winter and how many of these colonies were dead or reduced to a few hundreds of bees in spring, or had unsolvable problems (drone-laying, lack of queens).

At international level, honeybee colony losses are frequently reported and in the last years (2012-2020), the level of winter colony loss was reported almost annually (Bruckner et al., 2023; Gray et al., 2023; Steinhauer et al., 2023; Gray et al., 2020; Gray et al., 2019; Brodschneider et al., 2018; Kulhanek et al., 2017; van der Zee et al., 2016; van der Zee et al., 2014).

The overall winter loss rate was established in 2015/2016 at 12% (29 countries), in 2016/2017 at 20.9% (27 countries), in 2017/2018 at 16.4% (36 countries), in 2018/2019 at 16.7% (35 countries) and in 2019/2020 at 18.1% (36 countries), with significant differences between countries and years (from less than 5% to even more than 30%). At European Union level, the reported loss rates were as follow: 17.9% in 2017/2018, 14.5% in 2018/2019, and 17.7% in 2019/2020 (Gray et al., 2023; Gray et al., 2020; Gray et al., 2019; Brodschneider et al., 2018; van der Zee et al., 2016; van der Zee et al., 2014).

Romania was part of one of these studies (2019/2020), being reported an overall loss rate of 15% (CI 95%: 11.9-18.8%) ranked in the middle loss rate (Gray et al., 2023). The information was collected from 121 respondents (8298 colonies), representing less than 1% of total beekeepers in the country.

In a recent document published by the Ministry of Agriculture and Rural Development in Romania in the National Strategic Plan (2022, https://www.madr.ro) there are reported 32864 registered beekeepers who manage 2.352 mil. honey bee colonies. The larger apiaries with more than 150 colonies, who proved to register lower colony losses in Europe (Gray et al., 2020), are managed in Romania by 9.28% of total beekeepers (professional category), owning 26.9% out of the total number of colonies. This means that for almost ¾ of the colonies stock there are increasing risks to register colony losses, affecting the local honeybee diversity and beekeeping economy.

As multiyear data show, the questionnaire became an extremely important tool to collect useful information in a certain area or for a certain critical period. Its composition could change depending on the characteristics of studied population (country, practices, length of active season, etc.) or other interest variables (Bruckner et al., 2023; Gray et al., 2023). An increased rate of questionnaire completion and its regular application could help obtaining important information by analysis of data in time perspective (Brodschneider et al., 2022).

To collect general information on beekeeping and honey bee colony losses (depopulation and mortality), together with assessing the possible causes of them, different questionnaires were launched in the 2014-2023 period in Romania. Therefore, the objective of this paper was to perform a preliminary analysis of the collected data in order to assess the situation of colony losses in Romania and explain the possible causes. The obtained results offer a general view on Romanian beekeeping in the last years, data on the incidence of colony losses among the beekeepers, the mortality rate on subsets of validated questionnaires, as well as a synthetic evaluation of possible causes.

MATERIALS AND METHODS

Taking into account the multifactorial causes of colony losses from critical periods, generally incriminated worldwide, (e.g. varroa, phytosanitary treatments, nutrition) we chosen in our study a multi-questions survey (17-28 questions) in order to collect general information from beekeeping field combined with the estimation of colony losses and their possible causes in 2014-2023 period. The composition of questionnaire, the format (online/onsite) and evaluated periods were relatively variable, therefore, the data sets of respondents were selected and validated for certain questions of interest or period in order to be compared and statistically analysed. Following the validation process, the data on the number of questionnaires (respondents) for general questions about local beekeeping were recorded, from which respondents who provided data on registered number of colonies and associated loss were extracted for the calculation of the colony mortality rate (Table 1).

To evaluate the structure of respondents to a specific question and for a certain implementing period the response variable was represented by the total percentage of respondents.

To establish the overwinter mortality rate, the number of colonies registered and the associated colony loss were calculated for each validated questionnaire and set of questionnaires. In order to more accurately assess this indicator for the winter season 2022/2023, as the number of questionnaires validated for colony mortality rate calculation was low, we included a telephone interview conducted in 2023, on a subset of respondents from the online questionnaires. The loss rate of honeybee colonies expressed as mortality percentage during the winter period (November-April), was calculated by reporting the number of surviving honeybee colonies in the spring to the total number of colonies recorded at the beginning of winter (Bruckner et al., 2023; Gray et al., 2023, van der Zee et al., 2013). In the interview carried out in 2023 (71 questionnaires) we had the opportunity to re-evaluate and validate the loss rate for the winter 2022/2023 mortalities, as well as to evaluate the autumn mortality rate for August-October 2022 period, asking, case by case, information about possible causes.

The incidence of honeybee colony losses among beekeepers/apiaries was also reported as a percentage of respondents experiencing colonies' mortality or depopulation of more than 10%.

The results were analysed and presented tabularly or graphically for a better highlight of the multi-year situation. In this regard, the collected data were centralized through the Google survey platform and Excel Office files as well as analysed and synthetically presented by means of the NCSS 2021 Statistical Software, 2021.

RESULTS

1. Number of responding beekeepers

Table 1 presents the centralized data on the number of validated questionnaires for general information about beekeeping, as well as on the validated questionnaires for the calculation of the loss rate. For general questions the highest share of respondents (37.8%) was recorded in 2023 (278 questionnaires), followed by 30.9% in 2017, 12.9% in 2019, 11.6% in 2015 and 6.8% in 2016. Of these questionnaires, 58.3% were completed online, with the majority completed in 2023 (41.9%). The questionnaires were collected from all over the country (42 counties - Figure 1), but the validated ones for the loss rate were registered from 35 counties (including interview from 21 counties).

From the Table 1 and Figure 1 it can be seen that there is a relatively uneven distribution of respondents, both over the studied period and per country. It is important to mention here that in certain counties (Bacău, Dâmbovița, Gorj, Neamț, Sălaj, Sibiu, Timiș, Vaslui, Vrancea) a higher number of respondents was registered as a result of completing the questionnaires during the associative meetings, using the online or printed questionnaires (onsite).

In order to calculate the colony loss, only questionnaires that recorded accurate data on apiary size and winter mortality were used. For this purpose, 244 questionnaires (79.4%) of the total completed in printed format were validated. To check the obtained results in 2023, 71 beekeepers from the 2023 online data set of questionnaires, originating from 21 counties were interviewed. By reference to the published data on the total number of beekeepers, the percentage of participation in completing the questionnaire was generally below 1%. For example, compared to the most recent published data on the number of beekeepers in Romania (E.U. 2020-2022: n = 2316; https://agriculture.ec.europa.eu), the number of respondents who completed the questionnaire launched in 2023 was 1.2%, and in the case of validated questionnaires for loss rate the percentage was 0.57%.

Table 1. Number of validated questionnaires (respondents) by period, method of completion and number of participated counties

Survey	Validated questionnaires for general questions [Validated questionnaires for calculation the mortality rate of honeybee colonies]						
	2014/2015 (n)	2015/2016 (n)	2016/2017 (n)	2019 (n)	2022/2023 (n)	Total	
						(n)	%
Onsite	86 [67]	50 [49]	73 [66]	N/A	98 [62]	307 [244]	41.7 [77.5]
Online/*Interview	N/A	N/A	155	95	180 *[71]	430 *[71]	58.3 *[22.5]
Total	86 [67]	50 [49]	228 [66]	95	278 [133]	737 [315]	100
Number of participated counties	38 [19]	37 [8]	41 [9]	8	23 [21]	42 [35]	100 [83.3]

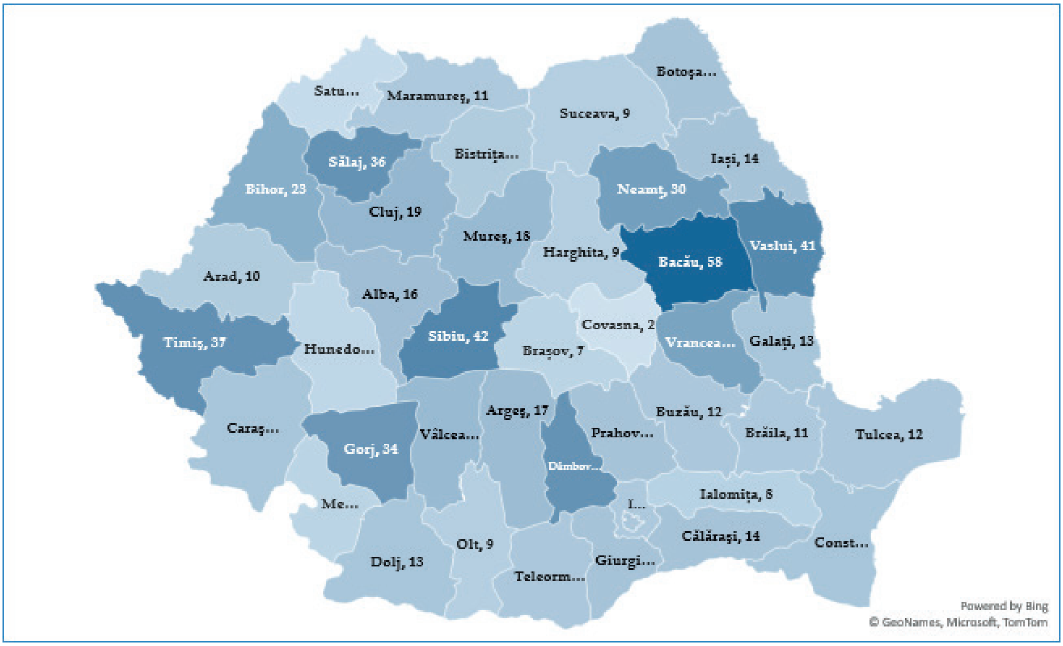


Figure 1. The map of the number of respondents' distribution (Romania, 2014-2023)

2. General data regarding beekeeping in Romania

Comparisons were made between data sets collected in 2019 and 2022/2023 when the questions regarding general data on beekeeping in Romania were included in the surveys.

2.1. Experience in beekeeping and age of respondents

From Figures 2a and 2b, it can be seen that the majority of respondents are experienced beekeepers (52.7% - 2019; 73.8% - 2022/2023), with over eleven years of activity, but relatively young, between 26 and 55 years old (75.9% - 2019; 60.5% - 2022/2023). In the case of the 2019 survey completed entirely online, the share of beekeepers with less experience (< 11 years) of the total respondents is higher (47.3%) compared to the results obtained by the combined way of completing (onsite and online), in 2022/2023, where the share of beekeepers with less experience decreases (< 11 years; 26.3%).

This is expected because the percentage of younger respondents (under 45 years; 34.6%) is lower in the results obtained in 2023 (Figure 2b) compared to 2019 (less than 45 years; 63.8%), a fact that indicates the different structure of the participants in the two social environments (online and combined). The situation is probably influenced by the lower participation of younger beekeepers with less experience in the associative beekeeping meetings, which especially contributed to the increase in the number of questionnaires collected in 2023.

These recent data can provide a better picture of the situation that could be extrapolated to the whole country.

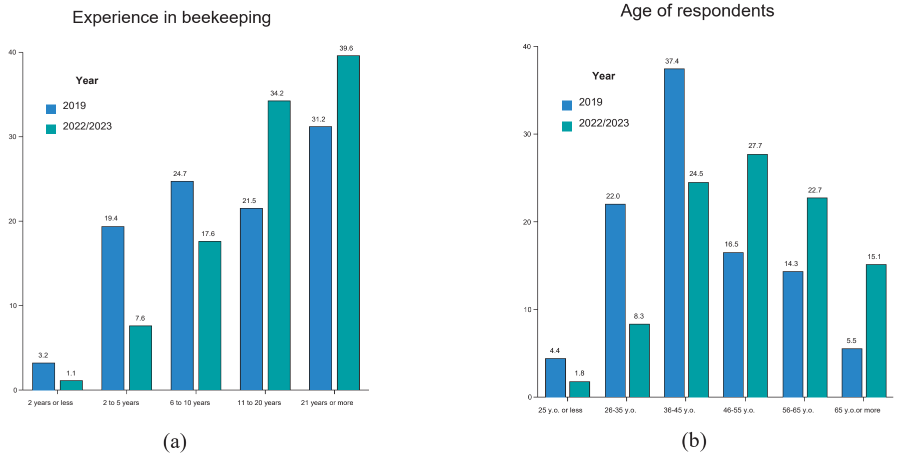
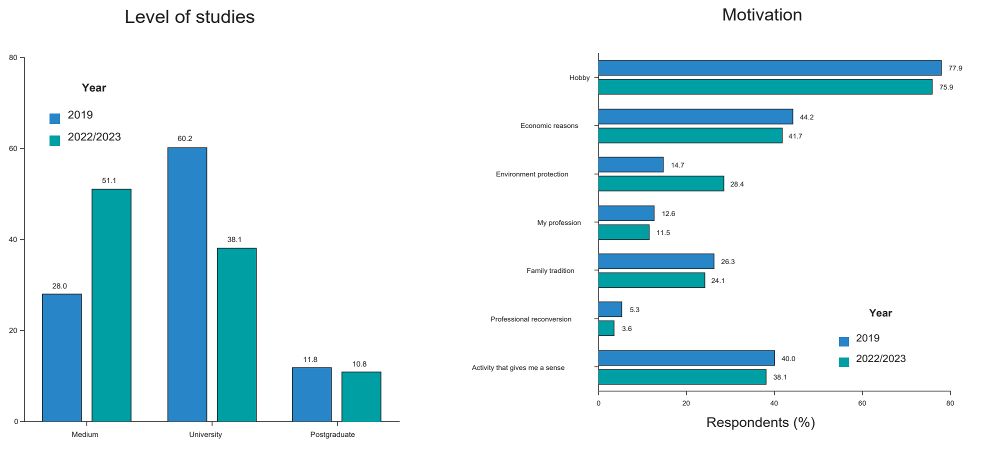


Figure 2. The structure of respondents (%) regarding the experience in beekeeping (a) and age of respondents (b)

2.2. Level of studies and beekeepers' motivation

Figure 3a gives the picture of the percentage of respondents with secondary education, lower in 2019 (28%) compared to 2023 (51.1%) and, as a result, the percentage of respondents with university education was higher in 2019 (70%), compared to 2023 (48.9%), but the proportion of respondents with postgraduate education was relatively similar in the two periods (11.8% in 2019 vs. 10.8% in 2023). Interestingly, the structure of the respondents regarding the motivation to practice beekeeping (Figure 3b), a question with several possible answers, was very similar in the two periods, except for the interest in environmental protection, which registered an increasing trend in the last period (2022/2023). It is noted that the majority of beekeepers practices beekeeping as a hobby (77.9% - 2019 vs 75.9% - 2023), for economic reasons (44.2% - 2019 vs 41.7% - 2023) and because it is an activity that gives them meaning in life (40.0% - 2019 vs 38.1% - 2023). "Family tradition" is an important reason for practicing beekeeping (around 25%), while "my profession" reason has only around 12%, and reconversion is quantified at around 4.5% of respondents.



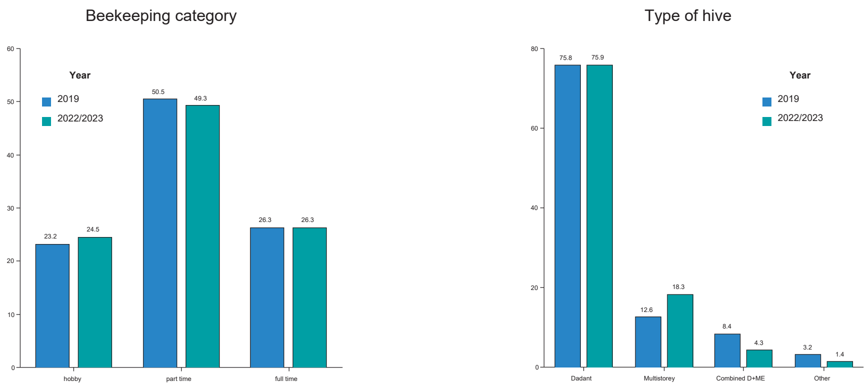
(a) (b)

Figure 3. The structure of respondents (%) regarding the level of studies (a) and their motivation in practicing beekeeping (b) in 2019 as compared with 2022/2023

2.3. Beekeeping category and the type of hive

Regarding the category of beekeeping practiced by the respondent beekeepers, an extremely similar structure of respondents can be noticed in both study periods (Figure 4a) - 2019 vs 2022/2023: approximately 26% - full time, 50% - part time, 24% - hobby.

With regard on the type of hive used (based on the type of frame used: Dadant, Romanian multi-storey hive, mixed or other type) an extremely similar structure of respondents can also be noticed (Figure 4b) between the two periods (on average approximately 75.85%, 15.45%, 6.35%, respectively 2.3 %).



(a) (b)

Figure 4. The structure of respondents (%) regarding the beekeeping category (a) and the type of hive used (b) (%) in 2019 as compared with 2022/2023

2.4. The size of the apiary and migratory beekeeping.

Regarding the size of the apiaries, by the application of General Linear Models (GLM), a normal probability of data was obtained throughout the period 2014-2023 (Shapiro-Wilk, Anderson-Darling tests), with significant differences between the three categories of apiaries (Table 2) as well as some variations between the structure of each period and different data sets.

If compared the data from two surveys (2016/2017 and 2022/2023) when the majority of respondents (68.7%) was recorded, significant differences were registered between all categories.

Table 2. Short descriptive statistics and multi-comparison test (Tukey-Kramer) on the structure of respondents (%) regarding the apiaries' size in the period 2014-2023

Size of the apiary	2014-2023*[1] Ave (%)	2014-2023*[2] Ave (%)	2014-2023*[1] C.I. 95%	2014-2023*[1] P-values	2016/2017 vs 2022/2023*[1] Ave (%)
Small (1-50 colonies)	31.7	22.54	25.4-38.0	0.0015 (L-S)	34.4
Medium (51-150 colonies)	55.5	64.76	49.2-61.9	0.0002 (M-S)	53.2
Large (Over 150 colonies)	12.7	12.70	6.4-19	0.0000 (L-M)	12.4

*Data reported to total respondents by [1]validated questionnaires for general data and by [2]validated questionnaires for colony losses reporting (see Table 1).

The Figure 5 shows the structure of beekeepers who practice migratory beekeeping. Taking into account that the average percentage of respondents (56.0%) for the whole period of time is relatively close to the average value of respondents' number (60.07%) for 2016/2017 and 2022/2023 surveys, when the highest percentage of completing the questionnaire was recorded, it is expected that this result will be a good indication of the real situation in the country.

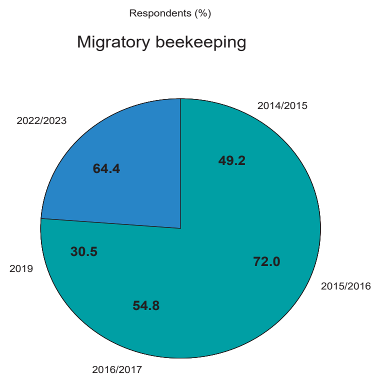


Figure 5. The structure of respondents' (%) regarding the migratory beekeeping in 2014-2023 period

3. Data regarding colony losses

3.1. Colony losses by overwinter mortality

The comparisons were made between data sets collected and validated in 2014/2015, 2015/2016, 2016/2017 and 2022/2023 (Table 1) surveys, totalising 315 questionnaires.

As shown in Table 3, it can be observed that the lowest honeybee colony mortality rate (Ave = 5.04%) was recorded in 2014/2015 and the highest (Ave = 17.04%) was recorded in 2022/2023 - interview and onsite data. It is also found that the rate of mortality assessed on the basis of the telephone interview is higher (Ave = 17.79%) than that calculated on the onsite (printed) questionnaires (Ave = 16.04%), the difference being probably due to the number of bee colonies, to the number of responding beekeepers, but also to the coverage degree of the country.

More than this, with the occasion of interview, the beekeepers were asked to offer information about the number of colonies in August 2022 in order to assess the colony mortality rate in autumn. The results show that a number of 7338 colonies were registered in August, with an average colony mortality rate of 7.82% [95% CI: 3.1-9.9] in autumn, which increases the total colony loss rate by mortality to 24.23% [95% CI: 18.5-29.4].

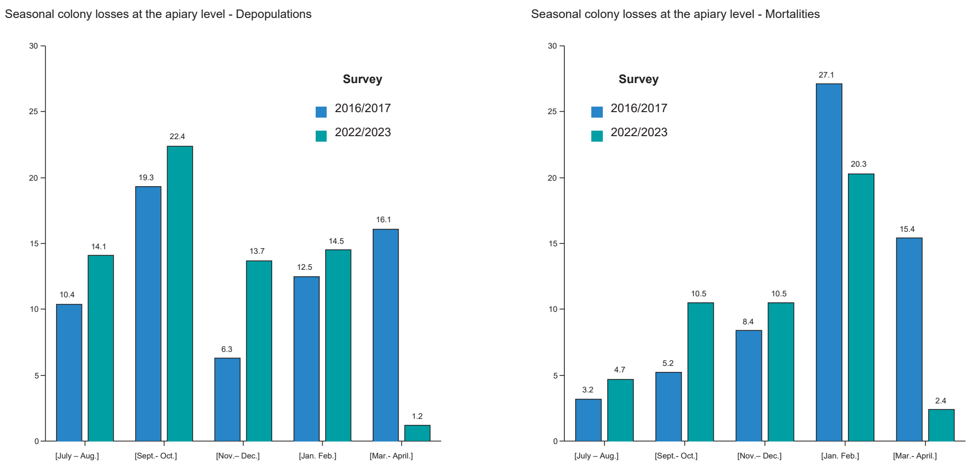
Table 3. The overwinter mortality rates of honeybee colonies in different surveys

Survey	Participated counties (n)	Validated questionnaires (n)	Registered colonies in autumn (n)	Registered colonies in spring (n)	Mortality of honeybee colonies over the winter (Ave, %) [95% CI]
2022/2023 (interview)	21	71	6570	5401	17.79 [14.4-25.2]
2022/2023 (onsite)	7	62	4969	4172	16.04 [8.2-19.7]
2022/2023 (total)	23	133	11539	9573	17.04 [13.2-21.0]
2016/2017 (onsite)	9	66	6145	5673	7.68 [5.7-14.3]
2015/2016 (onsite)	8	50	3848	3654	5.04 [3.1-10.9]
2014/2015 (onsite)	19	67	5739	5305	7.56 [4.3-10.3]
Total	35	315	27271	24205	11.24[9.8-14.1]

3.2. The incidence of colony losses

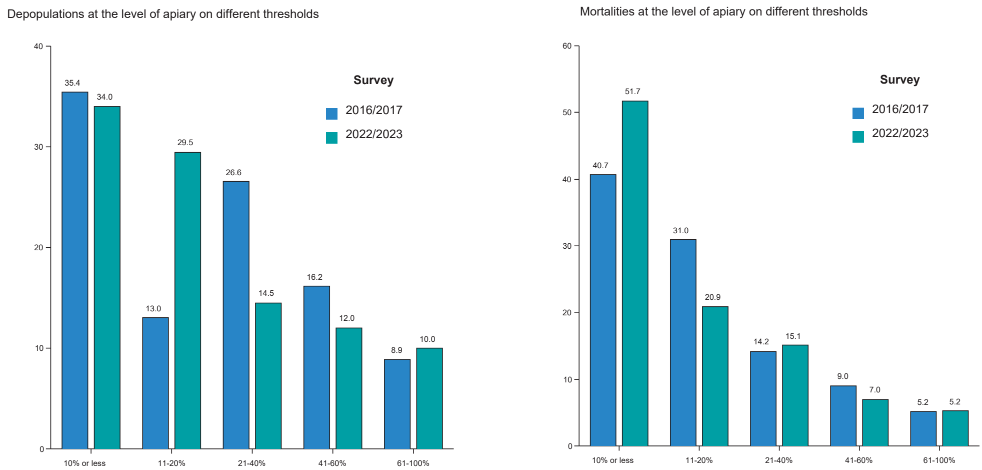
As can be seen from Table 4, the majority of beekeepers, on average 85.1% (min. 65.3% in 2015/2016, max. 93.2% in 2022/2023) declared that they had losses of honeybee colonies through depopulations and/or mortalities. However, through the subsequent questions, quantifying these losses on validated questionnaires, on average 56.5% of the responding beekeepers registered depopulations, located in one of the thresholds above 10% (min. 46.9% in 2015/2016, max. 69.7% in 2016/2017), while 32.7% of the surveyed beekeepers recorded colonies mortality above 10% (min. 18.4% in 2015/2016, max. 49.6% in 2022/2023). As regarding the evaluation of depopulation and mortality on various thresholds and months of the monitored period of the year (from July last season until April next year), the situation of questionnaires from the period 2016/2017 was compared with that of 2022/2023 when the largest number of questionnaires was collected (228, respectively 278).

The structure of respondents (%), depicted in Figures 6a and 6b, shows that the incidence of depopulations over 10% in 2016/2017 period was higher at the beginning of the monitored period (Sept./Oct.), but also at the end of it (Mar./Apr.), while the incidence of mortalities was higher during the winter period and early spring (Jan./Feb. and Mar./Apr.). In 2022/2023 survey, the incidence of depopulations was again higher in autumn (Sept./Oct.) as compared with early spring, while the mortalities were higher in winter (Jan./Feb.) and it is possible that they continued in March-April as the overwinter mortality rate was higher in 2023 as compared with 2017. It follows that the incidence of depopulations and mortalities was continuous, with the peak in September-October for depopulations and in January-February for mortalities. The overall incidence of depopulations and mortalities, using both, online and onsite questionnaires, on different thresholds, in 2016/2017 - 2022/2023 surveys, is presented in Figures 7a and b, but some differences in the collected data were registered in each survey for the whole period (2014-2023).



(a) (b)

Figure 6. The structure of respondents (%) who reported colony depopulations and mortalities over 10% at the level of the apiary, between July last season to April next season - a comparative situation 2016/2017 - 2022/2023. Note: Data for March-April 2023 is not accurate as a great part of the questionnaires was collected in February 2023



(a) (b)

Figure 7. The structure of respondents who reported colony depopulations and mortalities at the level of the apiary, on different thresholds, a comparative situation 2016/2017 - 2022/2023 from online and onsite surveys

Table 4. The incidence of colony losses in the last years in terms of beekeepers' percentage affected by different colony losses as reported to the total number of validated questionnaires

Survey	Beekeepers who declared colony losses as depopulation or/and mortalities		Beekeepers who declared depopulations over 10%		Beekeepers who declared mortalities over 10%	
	n	%	n	%	n	%
2022/2023 (interview)	66	93.0	44	62.0	39	54.9
2022/2023 (onsite)	58	93.5	33	53.2	27	43.5
2022/2023 (total)	124	93.2	77	57.9	66	49.6
2016/2017 (onsite)	58	87.9	46	69.7	13	19.7
2015/2016 (onsite)	32	65.3	23	46.9	9	18.4
2014/2015 (onsite)	54	80.6	32	47.8	15	22.4
Total	268	85.1	178	56.5	103	32.7

3.3. The possible causes of colony losses

Figure 8 presents a summary of data obtained by different surveys, regarding the most important reasons appreciated by beekeepers for recording colony losses. A number of 13 possible multiple causes were listed, with the possibility of registering a possible cause not listed under "others".

From the graphic representation, but also from the average recorded over the entire period regarding the number of respondents, neonicotinoid treatments at sunflower (50.5%), at rapeseed (38.6%) and in general those applied at the time of blooming (34.8%), together with varroosis (37.8%) are considered by beekeepers, major causes of colony losses.

Another important cause is represented by the quality of nutrition (34.1%) before entering the winter. Other possible causes were listed such as other diseases or pests (chalkbrood, American foulbrood, wasp attack - 4.8%), the lack of treatments with specific controlling products (5.7%), insufficient food storages for winter (9.4%) as well as of poor quality (7.1%), or a number of other causes mentioned by beekeepers (16.8%) such as: weather conditions, lack of honeyflows, lack of queens, theft, quality of artificial honeycombs or the humidity in the hives.

By collecting information on different factors in subsequent questions, there were registered valuable data which to be further analysed.

Using the Spearman correlation, a heat map (Figure 9) was produced to highlight the factors which are positively or negatively correlated each other or with the honeybee colony losses.

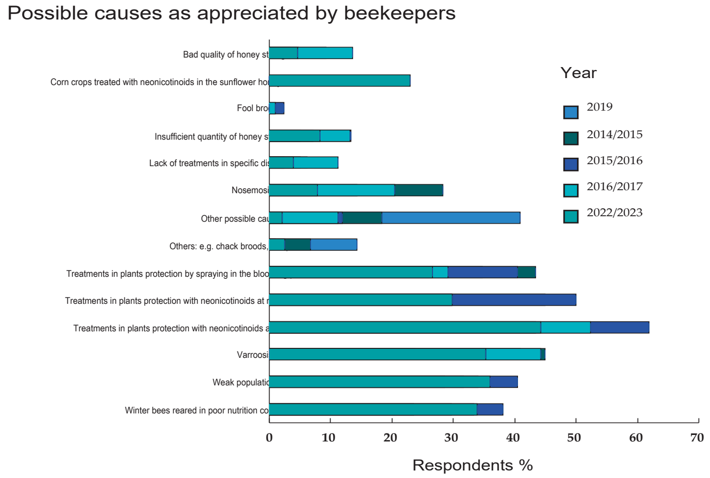


Figure 8. The structure of respondents (%) regarding the possible factors for colony losses registered in 2014-2023 period

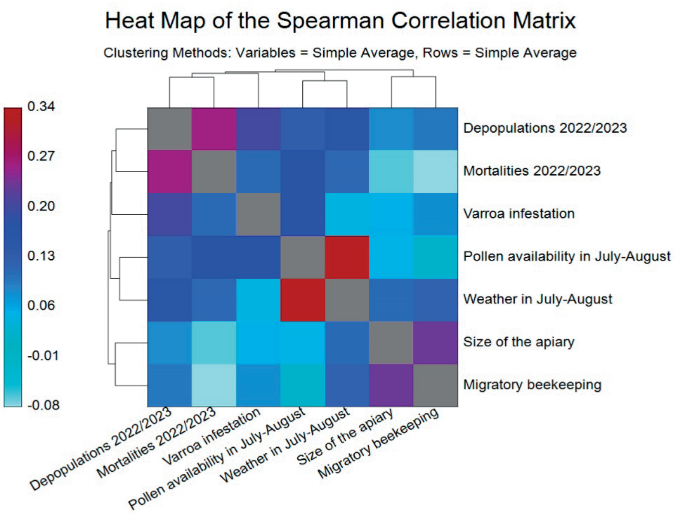


Figure 9. The Spearman correlations between different factors and colony losses expressed as depopulations and mortalities registered in the last survey - 2022/2023 (258 respondents)

DISCUSSIONS

Questionnaires are an important tool in beekeeping research, as they provide valuable information about this activity field. Completion depends on the degree of promotion of the questionnaire through social media or onsite, but also on the direct cooperation with beekeepers (Brodschneider et al., 2022). As the literature shows, the coverage degree of a country and response rate vary widely not only between countries and years (Gray et al., 2023), but also at national level, as resulted from our data (Figure 1). The number of responding beekeepers per survey in the present research is relatively low as compared with other countries that participated in COLOSS surveys in the same period 2014-2023 (Gray et al., 2023; Gray et al., 2020; Gray et al., 2019; Brodschneider et al., 2018; van der Zee et al., 2016; van der Zee et al., 2014). However, a higher rate of answers in online format was recorded in the periods 2016-2017 and 2022-2023 as compared with other data from Romania (Gray et al., 2023). Using more ways for distribution (online, e-mails, onsite) led, as expected, to increase the rate of completing, so the general view on the obtained results was improved, too. This was particularly noticed in the comparisons regarding general data on beekeeping, where younger beekeepers with less experience but with a higher level of studies were more responsive in online media (Figure 2a and b, Figure 3a). Surprisingly, the structure of respondents regarding the motivation to practice beekeeping, the beekeeping category and the type of hive used remains generally the same between the two data sets (Figure 3b, Figure 4a and b). Even if many similarities were registered, one can remark, by comparisons between these years (Figure 3b), that beekeepers are more and more engaged to practice beekeeping for reasons related to environment protection.

Regarding the size of the apiaries in Romania, data show that, in average for the whole period 2014-2023, the majority of beekeepers (55.5%) own “medium size” apiaries (51-150 colonies), being followed by “small” category (< 50 colonies) and “large” (> 150) apiaries. The data averages obtained for the whole period (Table 2) offer a good image that could be extrapolated to the total number of beekeepers in Romania. These data are different from those reported in the last published COLOSS survey in 2019/2020 (Gray et al., 2023), which show that the most part of responding beekeepers (90.2%) in 37 countries (26 countries belonging to EU) are part of the “small size” category of apiary.

The obtained answers at national level in all questionnaires show that migratory beekeeping is practiced in average by 56% of respondents, with greater variations between the years when smaller sets of answers (under 100) were received, but the total average being very similar with the years where a greater number of answers was collected. These data are contrasting with the results obtained in COLOSS questionnaires, where, overall, only 18.3% of respondents reported that they practice migratory beekeeping (Gray et al., 2023).

Regarding colony losses by overwinter mortalities, the results (Table 3) show a higher rate of colony losses registered in the last year (17.04%), as average of respondents’ number between onsite survey (16.04%) and interview (17.79%). This loss rate is higher as compared with the obtained ones in previous years at national level (under 10%) and in 2019/2020 Coloss survey for Romania (15%) (Gray et al., 2023), but relatively similar with the overall winter loss rate at the level of EU countries (17.7%), this being ranked in the middle category of colony loss rates. When these losses where quantified as incidence of colony losses on different thresholds (Table 4), 56.5% of respondents declared depopulations over 10% and 32.7% reported mortality over 10% on the all-validated questionnaires. The obtained data highlight also the variations of colony losses between different months. By comparing data form 2016/2017 and 2022/2023 (Figure 6) some critical periods were observed, as for example September-October for depopulations and January-February for mortalities (Figure 7). That is another reason why, as a case study, we have run the interview for the last survey, in order to find out also the autumnal colony mortalities rate, which was recorded at 7.82%, increasing the autumn-winter losses at a very significant level of 24.23%. These results come to complete the image of colony losses by mortality and depopulations in different period of the year, as a very complex phenomenon, with multiple causes, whose effects could overlap or cumulate (Gregorc, 2020; Hristov et al., 2020; Goulson et al., 2015).

Generally, the colony losses by depopulation are difficult to be quantified and understood because they are often a result of sublethal effects of different factors, as different chemicals who accumulate in the hive, pathogens or nutritional status (Martinello et al., 2020; Căuia et al., 2020; Cousin et al., 2019). The quantification of these type of losses in the same time with the identification of prevalent causing factors could contribute to a better awareness and monitoring in beekeeping management. With respect to the perceived causes of colony losses, the sets of answers show that the most part of respondents considers that colony losses are caused firstly by phytosanitary treatments at different crops, especially at sunflower and by varroosis (with the associated viruses), as well as by poor nutrition (Figure 8). These factors, singles or combined, as the mentioned literature shows, conduct to weaker colonies with low longevity honeybees in autumn and during the wintering season.

The questionnaire included also different questions on varroosis or nutrition (e.g. pollen and nectar flow availability in the late part of summer, food storage for winter), as well as questions to collect indirect information about nutrition (brood rearing and drone eviction in summer) which worth to be insighted by further analyses to predict the colony losses (Johannesen et al., 2022). They show a fluctuating picture from year to year, with difficulties in providing protein and carbohydrates the colonies need for daily requirements and winter storages. However, using a heat map for correlations matrix (Figure 9) between different factors and colony losses, one can notice that some factors are highly significant correlated (depopulations with mortalities, pollen availability with weather in July-August, migratory beekeeping with size of the apiary), some factors are middle values correlated (varroosis with depopulations, pollen with mortalities) and other factors are weakly correlated (mortalities with migratory beekeeping and size of the apiary).

It is important here to note that some information collected in the survey about the quality of last honey flows show that almost 2/3 of respondents rely on sunflower, this crop being extremely important in Romania for its impact on honey production (Ion et al., 2008; Ion et al., 2008; Ion et al., 2007; Stefan et al., 2008), as well as on the quantity and quality of honey storages for winter which affect the wealth of honeybees in the fall-winter season.

In the last period, around 10% of respondents reported a lack of the necessary honey flows to sustain the critical periods in the late season when the winter honeybees are reared, this being probably connected with climate changes. Regarding the weather conditions, 2022 year was appreciated by the responding beekeepers as the driest year in the monitored period (2014-2022), and this situation correspond to the data published by the Romanian National Meteorological Administration, which shows that 2022 was the driest year in the last 10 years and the third warmest year in the history of meteorological measurements in Romania (1900-2022), the average annual temperature being 11.77°C, with a thermal deviation of 1.55°C compared to the average of the period 1981-2010. In fact, six years in the surveyed period (2014, 2015, 2018, 2019, 2020, 2022) were also among the warmest 5 years from 1900-2022 in Romania, with 2019 the warmest year, and the 2012-2022 interval represents the warmest period in the history of measurements, a fact that can have important repercussions on honey flora and beekeeping. Weak population for winter and wintering bees reared in poor nutrition following unfavourable weather conditions were between the biggest concerns of respondents (Figure 8).

The reported here colony losses data and the possible factors affecting colony welfare could help different stakeholders to prioritize further decisions and researches to prevent these phenomena and to support beekeepers to counteract colony losses and adapt to new challenges related to climate change and other risk factors.

CONCLUSIONS

It is for the first time that a temporal situation on honeybee colony losses in Romania is presented. The obtained results on general beekeeping data and colony losses, both depopulations and mortalities, show a very dynamic and complex situation, which requires further evaluations. Using regularly a standardised, complex questionnaire, adapted to local conditions and requirements, will ensure the obtaining of useful and comparable data at national and international level.

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PRELIMINARY ANALYSIS OF A BEE POLLEN SAMPLE COLLECTED DURING AUTUMN IN THE CORNETU AREA (ILFOV COUNTY)

Mirela DRĂGAN[1], Monica ENACHE[1], Silvana Mihaela DĂNĂILĂ-GUIDEA[1, 2], Ștefana JURCOANE[1, 2, 3]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania
[2]Microbial Biotechnology Centre - BIOTEHGEN, 59 Marasti Blvd, District 1, Bucharest, Romania
[3]Academy of Romanian Scientists, 54 Splaiul Independentei, 050094, Bucharest, Romania

Corresponding author email: mirela.dragan78@yahoo.it

Abstract

The present study describes the results of a pollen analysis carried out using light microscopy of a sample of bee pollen collected from a stationary apiary situated in the commune of Cornetu (Ilfov County) in October 2022, during a period with low temperature during the night, and after a summer characterized by very hot weather and severe drought conditions. To determine the preference of pollen sources by the honey bee colony, identification of melliferous plants in the apiary vicinity and up to the periphery of the commune of Cornetu was carried out. Cultured crops such as artichoke, buckwheat, cabbage, common vetch, mustard, phacelia, rape and several autumn-flowering garden plants were blooming at the moment when the current study was carried out. Also, uncultivated areas colonized by allergenic plants were identified in the foraging area around the beehive. After sorting the pollen pellets based on colour, the results indicated several origins of pollen, including pollen of Brassicaceae, Asteraceae, Rosaceae, Portulaca and also pollen of the highly allergenic anemophilous species ragweed - Ambrosia artemisiifolia, known as a popular source of pollen for bees.

Key words*: autumn pollen; bee pollen; optical microscope; pollen morphology, stationary apiary.*

INTRODUCTION

The development of rural areas through bee colonies and pollination activities could ensure the balance of the agricultural ecosystem in order to conserve environmental resources.

Romania ranks second in the European Union, in terms of the number of beehives of bee families and first in the production of bee honey (European Commission, 2022; European Parliament, 2018). With a view to the rural development of the beekeeping sector in Romania, a particular importance is aimed at intensifying the potential of beekeeping determined by the pedoclimatic and melliferous base conditions in Romania.

The melliferous potential of Romania includes a series of plants from the spontaneous or cultivated flora, which through nectar and/or pollen secretions provide bees with the raw material necessary for the maintenance and development of bee families.

By flowering from February to March and until the end of October, melliferous plants from the S-W region of Ilfov County provide bee families with pollen and nectar throughout the active beekeeping season.

In addition to the melliferous sources mentioned above, bees also collect manna (honeydew) during certain periods of the active beekeeping season, this frequently occurs in the case of forests (deciduous and coniferous).

As a honey source is also considered the highly concentrated sugar secretion (50-80%) of flower buds and fruit bushes before the opening of flowers, with impact on the development of bee colonies (Iordache et al., 2008). The plants that bees search for nectar, manna or pollen form together the melliferous base (Beekeeping Law no. 383/2013 updated 2020 - Romania).

In an article, Spulber et al. (2020), pointed out the need for a study of the pollen types and their antioxidant properties for the whole geographical area of Romania.

The country's climate is mainly temperate-continental with excessive nuance, with hot and dry summers and cold winters, dominated by the frequent presence of cold continental air masses from the East or Arctic air from the North and strong blizzard winds, the average annual amount of precipitation is 460-500 mm.

The current research describes the results of a pollen analysis carried out using light microscopy of a sample of bee pollen collected from a stationary apiary situated in the commune of Cornetu (S-W of Ilfov County, Romania) in October 2022, during a period with low temperature during the night, and after a summer characterized by very hot weather and severe drought conditions (Figure 1), as part of a larger study that monitors both the melliferous power of the local crops and the amounts of honey and pollen obtained per hive.

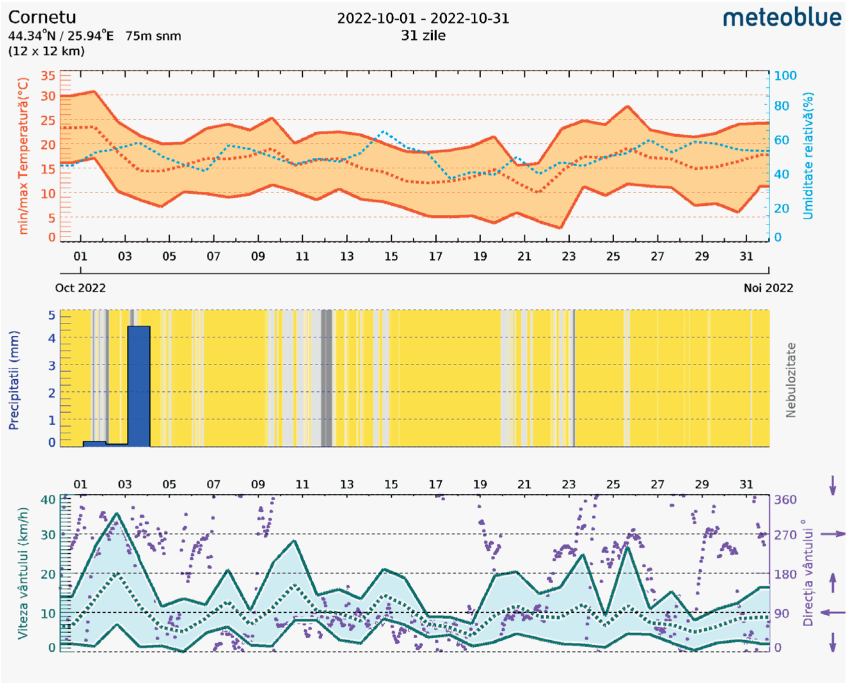

Figure 1. Meteorological records (temperature, precipitation and wind speed) in Cornetu, Ilfov County, Romania, October 2022 (Source: Romanian weather archive - retrieved from https://www.meteoblue.com/ro)

MATERIALS AND METHODS

Buckwheat (*Fagopyrum esculentum* “Zita”), mustard (*Sinapis alba* “Maryna”), phacelia *(Phacelia tanacetifolia* “Stala”*)*, common vetch (*Vicia sativa*) and camelina (*Camelina* sp.) crops were sown in July 2022, at the location of the apiary used in the current study, in the commune of Cornetu located in the southwest of Ilfov County, on the left bank of the Argeș river (where it forms the Mihăilești reservoir, Giurgiu County) on Sabar riverside with an area of 17.12 km².

Ilfov County is located in the south-southeast part of Romania, in the center of the Romanian Plain. Like a protective wall that surrounds a fortress, the county extends around the capital of Romania, Bucharest, being surrounded in turn by the neighboring counties of Prahova to the north, Dâmbovița to the west, Giurgiu to the southwest, Călăraşi to the southeast and Ialomița to the east (Figure 2).

In Ilfov, the agriculture occupies an important place, having great reserves and possibilities for development determined by the qualities of the soils, climate factors, technical and material endowments and the adequate labor force.

Approximately 95% of the agricultural potential of Ilfov county is held by arable land, the remaining 5% being occupied by trees, vineyards, meadows and hayfields (rasfoiesc.com, 2023).

Due to the geographical positioning of Ilfov county in the southern part of the country, there are relatively varied natural conditions, which are also reflected in a variety of soil types (Stănilă & Parichi, 2011).

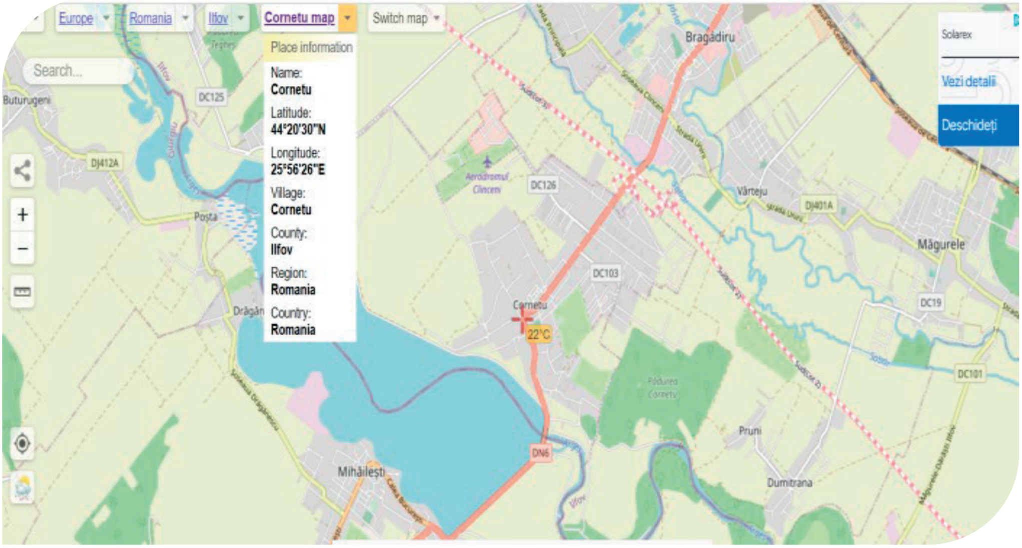

Figure 2. The location of Cornetu, S-W of Ilfov County, Romania
(Source: Map retrieved from https://cornetu-if.pe-harta.ro/)

To determine the preference of pollen sources by the honey bee colony, identification of melliferous plants in the apiary vicinity and up to the periphery of the commune of Cornetu was carried out.
The apiary in the commune of Cornetu consists of 25 bee families, horizontal hives, with a capacity of up to 20 frames 1/1. This type of hive is very easy to maintain and use. The apiary is of a stationary type located on extra-urban land with a large proportion of spontaneous vegetation.
Figure 3 illustrates the activities of mechanized processing by plowing and shredding the plots of land intended to be sown with buckwheat, mustard, phacelia, common vetch and camelina in spring 2022.

Figure 3. Ploughing at the location of the apiary used in the current study, in the location commune of Cornetu, Ilfov County, Romania (10.03.2022)

To obtain information on what flowers the bees are visiting at different points in the season, beekeepers can use pollen traps on hives, and collect pollen samples at regular intervals

(Waters, 2014). In the present work pollen was collected in October 2022 (for the period September-October 2022) and was analysed at the Laboratory of Biology of the Faculty of Biotechnologies, from University of Agronomic Sciences and Veterinary Medicine of Bucharest, using the method and equipment described previously (Enache et al., 2019). In addition, a digital S-Eye 2.0 microscope camera was also used. For the present study, the pollen descriptions were compared to those found in the literature, including: Diethart & Heigl (2020); Halbritter (2016); Halbritter et al. (2020a; 2020b); Heigl (2020); Sam et al. (2020); ScientificBeekeeping (2023); Stebler (2023); Şerbănescu-Jitariu et al. (1994); Tarnavschi et al. (1981; 1987; 1990).

RESULTS AND DISCUSSIONS

Cultured crops such as artichoke (*Cynara* sp.), buckwheat (*Fagopyrum esculentum* "Zita"), cabbage (*Brassica oleracea*), common vetch (*Vicia sativa*), mustard (*Sinapis alba* "Maryna"), phacelia (*Phacelia tanacetifolia* "Stala"), rape (*Brassica napus*) and autumn-flowering garden plants (dahlia - *Dahlia* sp., common zinnia - *Zinnia elegans*, pendant amaranth - *Amaranthus* sp.) as well as dog rose species and park rose varieties were blooming at the moment when the current study was carried out. Also, uncultivated areas colonized by allergenic plants were identified in the foraging area around the apiary.

After sorting the pollen pellets based on colour, the results indicated several origins of pollen, as follows: pollen pellet colour - yellow, pollen: tricolpate, prolate shape, with medium size (P = 35-40 μm, E = 27.5 μm), reticulate ornamentation, thick sporoderm (Figure 4), the images are similar to those of the pollen of white mustard var. Maryna (Gardenseedsmarket.com, 2023) collected in Cornetu and analysed in the laboratory previously (Figure 5).

Pollen pellet colour - black/dark brown, pollen: tricolporate, oblate-spheroidal shape, with medium size (P = 40-45 μm, E = 45-50 μm), echinate ornamentation, the surface of echini has granules, possible ruderal Asteraceae (Figure 6).

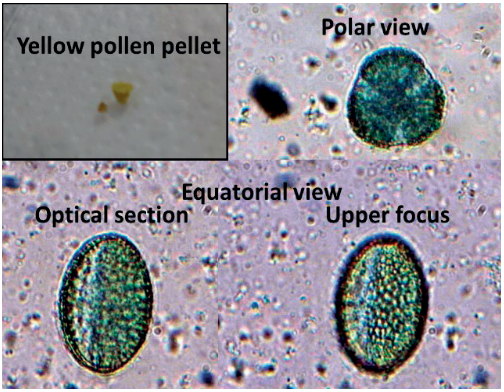

Figure 4. Yellow pollen pellet showing tricolpate pollen (Brassicaceae)

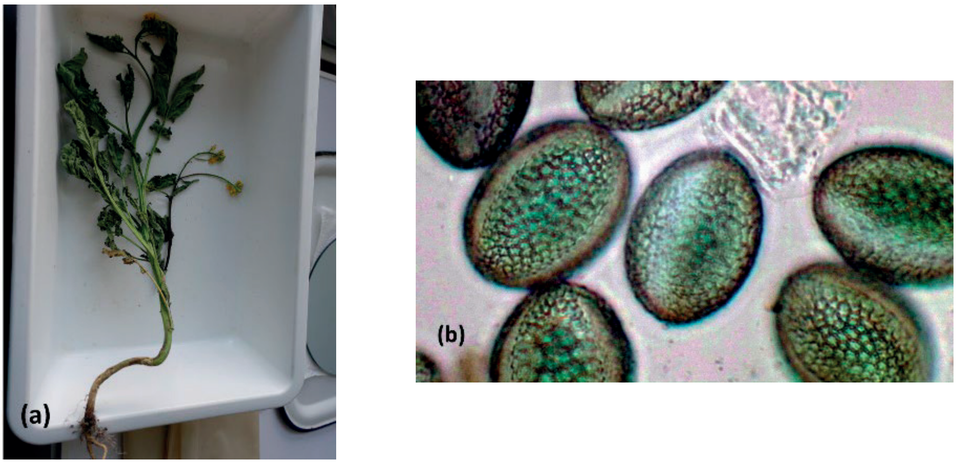

Figure 5. (a) *Sinapis alba* "Maryna" from the commune of Cornetu and (b) its pollen, 29.09.2022

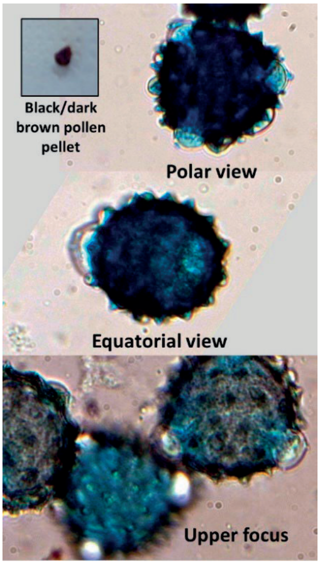

Figure 6. Tricolporate echinate pollen of medium size, possible ruderal Asteraceae

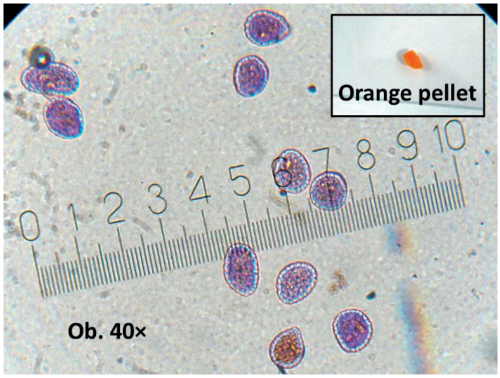

Figure 7. Rust fungi, fresh mount of pellet, toluidine blue stain

Pellet colour - orange, spores of Pucciniales (rust) fungi (fungal parasites that grow on some plants) (Figure 7) - they are sometimes collected by bees.

Pollen pellet colour - beige, pollen: tricolporate, suboblate shape, with medium size (P = 25 µm, E = 30-32.5 µm), psilate surface, possible Rosaceae pollen (Figure 8).

Pollen pellet colour - yellow, pollen: triaperturate, echinate, fenestrate, with medium size (37.5-40 µm), possible ruderal Asteraceae (Figure 9).

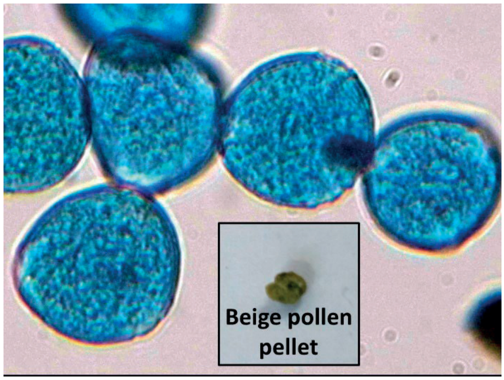

Figure 8. Tricolporate, suboblate, medium-size pollen, possible *Rosa* sp.

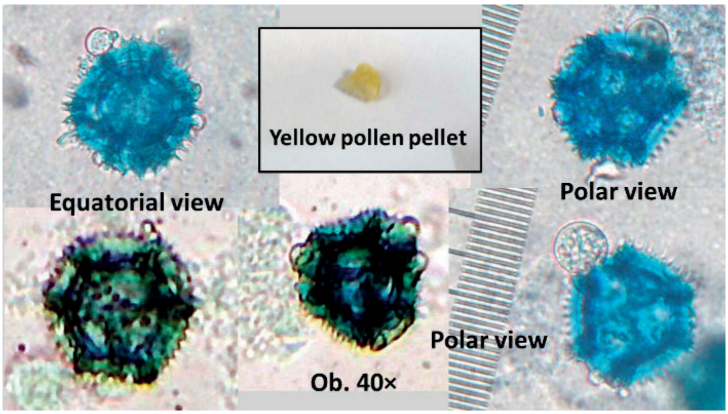

Figure 9. Triaperturate, echinate, fenestrate, medium-size pollen, possible ruderal Asteraceae

Pollen pellet colour - orange, pollen: pantocolpate, spheroidal, with large size (51-62 µm), echinate, with granular cytoplasm and pollenkitt (Figure 10), possible *Portulaca grandiflora* (Portulacaceae) that is traditionally found in villages all around Bucharest, since they are easy-care self-seeding plants, very pretty and drought resistant.

Pollen pellet colour - yellow, pollen: triaperturate, spheroidal, echinate, spines rather flat with a large base, with small size (13-16 µm), no pollenkitt (Figure 11), possible pollen of the highly allergenic anemophilous species ragweed - *Ambrosia artemisiifolia* (Asteraceae), that is found in the apiary vicinity, and is known to be a popular source of pollen for bees.

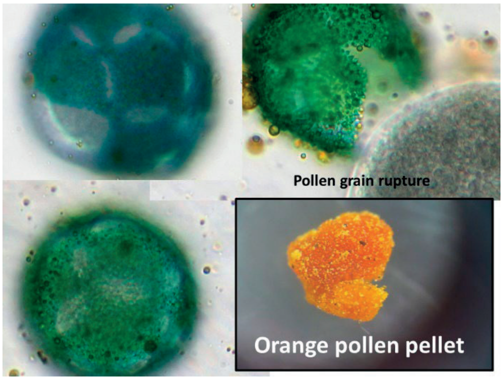

Figure 10. Pantocolpate, spheroidal, echinate, large-size pollen with granular cytoplasm, possible *Portulaca grandiflora*

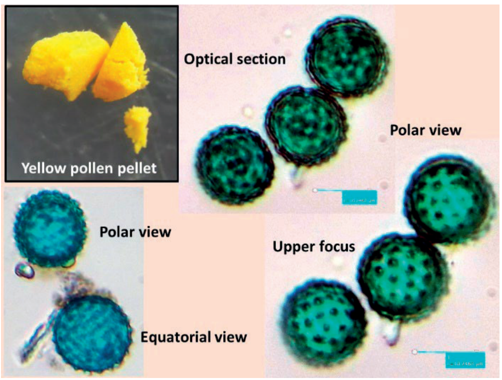

Figure 11. Triaperturate, spheroidal, echinate, small-size pollen with no pollenkitt, possible ragweed pollen

Pollen pellet colour - beige, pollen: periporate, pori with aperture membrane ornamented, spheroidal shape, with small size (14-22 µm) (Figure 12), possible pollen of amaranth (*Amaranthus* sp., Amaranthaceae), a cosmopolitan genus with ruderal species and species that are cultivated as leaf vegetables, pseudocereals, and ornamental plants. Similarly with *Ambrosia* pollen, the pollen of *Amaranthus* is highly allergenic.

Pollen pellet colour – orange-yellow, pollen: tricolporate with echinate surface with ~6 µm long echini, medium size spheroidal pollen (Figure 13), similar to sunflower pollen (*Helianthus annuus*), but found in other Asteraceae as well.

Figure 12. Periporate, spheroidal, small-size pollen, possible pollen of amaranth

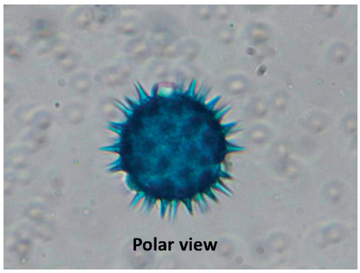

Figure 13. Tricolporate medium-size pollen with echinate surface, possible Asteraceae

CONCLUSIONS

The aim of the current research was to determine the food resources available to the bee families of the apiary, in the climatic conditions of prolonged autumn manifested in Cornetu in the year 2022. Currently, great importance is given to beekeeping due to the special role that the bee has in preserving biodiversity, therefore special efforts are still required to protect this insect from the aggression of external factors such as pollution, climate changes and various disruptive human activities (such as unregulated phytosanitary treatments or herbicide excess).

The increasingly diverse aspects faced by beekeepers in the practice of this noble activity require complex and diversified information on the problems existing in the new socioeconomic conditions, but also regarding the environment.

The current results indicated several origins of pollen, including pollen of Brassicaceae, Asteraceae, Rosaceae, *Portulaca* sp. and also pollen of two highly allergenic anemophilous species: ragweed and amaranth.

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STUDIES ON THE DYNAMICS OF THRESHING APPLIANCES OF CEREAL HARVESTING COMBINES

Anișoara Claudia DUMA COPCEA, Casiana Doina MIHUȚ, Lucian Dumitru NIȚĂ, Daniela Nicoleta SCEDEI, Okros ADALBERT, Sorin BUNGESCU, Lia Sanda ROTARIU, Constanța Corina SÎRBU

University of Life Sciences "King Mihai I" from Timisoara, 300645, 119 Calea Aradului Street, Timisoara, Romania

Corresponding author email: anisoaradumacopcea@usab-tm.ro

Abstract

The present paper presents a comparative study of the threshers that equip different types of self-propelled combines for cereal harvesting. Particular attention is paid to the problems related to the exploitation of the threshers, focusing on some more important aspects, including those regarding the technological adjustments, the organization and management of the harvesting chain, the execution of the harvesting works and their quality control. We measured the constructive and functional parameters of each threshing device and determined the hourly productivity of each combine and the consumption of diesel per ton of harvested wheat. This shows that worldwide harvesting combines tend to use double-flow threshers with tangential flow due to the following advantages: both threshing and separation of the grains from the straw are done, both length of the thresher and vibrations are reduced because there is no shaking for separation, the percentage of non-threshed ears is reduced by the fact that the material is trained in rotary motion inside the threshing device.

***Key words**: thresher, self-propelled combines, harvesting works, constructive parameters, technological adjustments.*

INTRODUCTION

Combine harvesters in use today can achieve production rates of over 70 t/h of grain; however, their technical performance potential is currently, by no means, exhausted. This paper describes the global combine harvester market, its inventories and recent productions. According to the distribution of farm size and regional field sizes, Germany was divided into three combined cropping regions. Simulation results show that about 45,000 units of agricultural combines of three performance categories are needed in Germany. However, calculations show that future domestic sales of combine harvesters will largely depend on the lifetime of the units (Clemens et al., 2015).

As stated by Kutzbach & Quick (1999), the main processes in a modern harvester are gathering and cutting, threshing, separating, cleaning, and material handling. Currently, designers of the combine harvesters pay more attention to the improvement of the quality of the control process and of its automatic control, and they improve the trafficability of the chassis and the environment protection. They argue that an increase in engine power increases the throughput of the combine harvester. (Kutzbach & Quick, 1999). Combine harvesters with axial threshing-separating device are, after design improvement of the axial drum, increasingly used for the harvest of the grain crops and rape with increased grain moisture content (Špokas, 2005; Špokas, 2006). Currently, the combine harvesters equipped with the straw walker with medium capacity are prevailing. Grain losses and grain damage are used as basic indicators for the evaluation of their technological function (Eimer, 1988; Feiffer et al., 2005). The working capacity of conventional grain harvesters is mainly determined by the working capacity of the tangential threshing machine.

Its working capacity depends on both the technical and functional characteristics of the actual tangential threshing machine and the biological characteristics of the harvested plant mass (Chirilă, 2012). The piece of material driven by a rail of the beater is rolled in the threshing space and, under the action of frictional and centrifugal forces, the material is combed and the seeds are separated (Ivan & Vlăduț, 2014). What mainly characterises the major differences in the technological flows of the current grain harvesters is the type of systems for picking up the material from the field and the construction of the threshing bodies. The vast majority of combines currently use traditional (tangential) threshers with a single beater, but the current trend is to use combines equipped with axial threshers or with multiple beaters/threshing rotors and separation, which aim to increase working capacity.

According to the technological flows, the combines are divided into two categories:

-Combines with tangential threshing machines, where the transverse threshing machine is located immediately after the central conveyor, the material flow entering perpendicular to it, and the material taken from the screw conveyor and placed in the centre where the threshing machine is located hits the bars (rails) beating roughly tangentially;

-Combines with axial threshing machines, where the thresher is located axially on the longitudinal axis of the thresher and, in some cases, the transverse flow of the material moves through the thresher on a helical path, and the material is separated mainly due to the friction and forces of the centrifuges and less to collision (Chiril et al., 2011).

The threshing device of conventional grain harvesters is of the tangential type.

This is the main working organ of these combines in terms of seed separation and energy consumption (Kutzbach, 2003).

The tangential threshing device is positioned in the technological flow of the conventional grain harvester between the central elevator and the shaker.

The axial thresher is a more recent achievement, being introduced in the construction of grain harvesters in 1970, especially in the construction of grain corn harvesters, later extending to straw grain harvesters as well (Ivan, 2009).

Under optimal conditions, the separation of seeds in the tangential thresher can reach a maximum of 85%, falling below 50-60% in case of improper adjustments and of a crop with high humidity (Miu & Kutzbach, 2008).

The tangential thresher must ensure a threshing process with unthreshed seeds and remaining in ears below 1%, free seeds in straw below 14% and damaged seeds below 2% of the harvested production (Segărceanu, 1990).

In a tangential feed axial threshing unit, the grains begin to separate through the cage openings as they become free.

Such threshing units are used in combines designed by the German manufacturer CLAAS® that are used worldwide (Miu, 1995).

In this context, the scientific organization of the harvesting of grassy cereals can result in a shortening of the harvesting period by 20-25% and an increase in labour productivity by 24%.

In order to achieve these targets, as well as for the rational operation of the combines, it is necessary to take a series of organizational measures: preparing the field for harvesting, choosing the methods of moving the combine in the field, and ensuring the means of transport.

Threshing is the most important function of grain harvester.

Grain loss and damage in harvesting are significantly related to threshing theory and technology.

There are four kinds of threshing principles including impact, rubbing, combing and grinding.

Four types of contact models between grain and threshing components have been constructed correspondently.

Grain damage can be regarded as a function of peripheral velocity and contact pattern of impacting.

Grain loss can be regarded as a function of contact pattern of rasp bars.

Grain loss coming from cleaning and separation in the subsequent process of combing threshing was significantly decreased.

Tangential and axial threshing technologies have been applied in grain threshing system widely.

It showed that in the combined application, tangential rolls are used to accelerate grain flow, and axial rolls are used to increase threshing quality especially lower loss and damage.

Conical concave may take the place of the traditional cylindrical one. With the development of sensor technology and communication technology, intelligent harvesting robot and automatic threshing system will be integrated together to improve grain quality and operation comfort (Jun Fu et al., 2018).

The statistic proposes the analytical provisions and the experimental results of the throughput capacity of the threshing apparatus of grain harvesters, from the capacity of straw, grain, straw content, blockage of design characteristics.

The threshing capacity is most significantly affected by weediness due to humidity, with a relative weediness of 0.05 bread weight, the capacity of the threshing machine is reduced by 2.5%, and with 50% weed by 16%.

Loading of the threshing machine determines the productivity of the combine and fuel efficiency due to fuel consumption for harvesting 1 hectare, as well as threshing 1 ton of crop.

The power of the combine engine is designed to perform work in extrem econditions, as a result, the average loadis 2/3 of full.

During the operation of the combines, the time is only 45-50% of the total operating time of the engine. Underemployment of the engine leads to overconsumption of fuel.

So, if the SMD-21M diesel at full operational power of 103 kW consumes fuel of 0.24 kg on 1 kW for an hour, at loading on 50% 51 kW s. specific fuel consumption increases by 41 g/kWh, or 17%, which corresponds to fuel consumption 2.1 kg/year (Rogovskii et al., 2020). The technological process of separation of the grain pile from large impurities (spikelets, straw remnants, etc.) and light impurities (husks, small weed seeds, etc.) in combine harvesters (CH) occurs in the cleaning system by air-sieve.

The existing air-sieve cleaning systems have a number of significant drawbacks as to the quality of the implementation of the technological process of separation of the grain pile from impurities. This is due to the increase in throughput, uneven ripening of the grain mass, unevenness of grain entering the cleaning, different concentrations of grain particles (grain and impurities), moisture and grain contamination, uneven distribution in width when harvesting on slopes. This is due to the unevenness or violation of the uniform distribution of the air flow over the entire area of the sieves due to the imperfection of the existing structures of the cleaning system, as well as the lack of a complete theoretical description and methods to substantiate their structural and technological parameters (Mudarisov et al., 2017).

MATERIALS AND METHODS

For the study of the dynamics of threshing machines, two constructive types of self-propelled combines for harvesting straw grain were chosen - one with a tangential threshing machine (with beating rails) and one with an axial threshing machine.

These studies were carried out at agricultural farms in Timiș County (Carani, Bărăteaz) and Caraș-Severin County (Nicolinț), both in Romania.

The constructive and functional parameters of each threshing machine ere measure to determine the hourly productivity of each combine and diesel consumption per ton of harvested wheat.

The self-propelled combines on which these studies were carried out are:

- The Claas Lexion 750 self-propelled combine harvester;
- The John Deere S 670i self-propelled combine.

RESULTS AND DISCUSSIONS

The Claas Lexion 750 self-propelled combine harvester is equipped with a Mercedes-Benz OM 470 LA diesel engine with a total displacement of 10.7 l.

The diesel tank has a volume of 800 l, and the engine complies with Tier 4f pollution standards. The threshing machine of this combine is of the APS Hybrid type, with pre-separation accelerator.

The advantages of this threshing system are the following:

- The plants harvested and transported to the thresher first reach the pre-accelerator, which increases the material supply speed of the threshing machine from 3 m/s to 12 m/s;
- The speeds of the three drums (accelerator, beater, and post-beater) can be adjusted independently depending on the threshing conditions;
- The harvested material moves at a higher speed between the rotors;
- The centrifugal forces increase due to the increased speed of the material, which helps

separate the grains from the threshed ears faster;
- The beater's load is reduced by 30% because part of the grains is threshed by the pre-separation rotor;
- The closed beater of the tangential threshing machine ensures smooth threshing for any type of culture;
- The feed flow of this threshing system increases by about 20% for the same diesel consumption;
- It is the most efficient threshing system for the cultivation of wheat.

The measurements of the threshing system of this combine were processed, interpreted and centralized in Table 1.

Table 1. Measurements obtained on the Claas Lexion 750 self-propelled combine harvester

Parameter	UM	Value
Beater diameter	mm	600
Beater width	mm	1420
Beater speed	rot./min	450-1050
Beater angular speed	rad./sec	46-108
Centrifugal acceleration	m/s^2	635-3498
Thresher cinematic index	K	64-357
Counter-beater wrapping angle	degrees	142
Counter-beater area	m^2	1.45
Thresher feeding flow	kg/sec	22
Hourly combine productivity	t/h	48

The John Deere S 670i self-propelled combine is equipped with a 465 HP diesel engine.

The axial threshing device of this combine has a single rotor "Single Tine Separation - STS" and fulfils both the role of threshing and that of separating unthreshed seeds and ears from the top of the material.

The rotor of the threshing machine, of a special construction (bullet rotor), is equipped with beating bars on the front section and with separation fingers on the rear section.

The separation cylinder on the outside of the rotor is divided into three concentric sectors, the first sector having the smallest diameter and the last sector having the largest diameter.

The concave separation grates are very easy to replace when changing from one crop to another.

Due to the concentricity of the three separation sectors, the material is driven tangentially and longitudinally in the separation space.

The gradual opening of the separation diameter allows the flow of material to move by expansion from the entrance to the exit of the separation "cage".

The separation surface is larger, the centrifugal forces are increasing, the movement of the rotor is continuous and uniform and, as a result, the quality of the process of separating the grains from the straw is superior compared to the classic separation systems.

Thanks to this special construction, the material does not agglomerate inside, the evacuation from the rotor is relatively easy, and there is, practically, no possibility of clogging the device with material.

Due to the expansion movement of the material, it is energetically loosened and, therefore, harvesting is possible even at higher humidity. Also, due to the increase in centrifugal forces, the process of forced separation of the grains from the straw intensifies.

The technical characteristics of the threshing machine of the John Deere S 670i self-propelled combine are presented in Table 2.

Table 2. Measurements obtained on the John Deere S 670i self-propelled combine

Parameter	UM	Value
Rotor diameter	mm	762
Rotor length	mm	3124
Rotor speed	rot./min	210-1000
Rotor angular speed	rad./sec	22-105
Centrifugal acceleration	m/s^2	193-4410
Thresher cinematic index	K	20-448
Counter-beater wrapping angle	degrees	124
Counter-beater area	m^2	1.54
Thresher feeding flow	kg/sec	21
Hourly combine productivity	t/h	42

In this paper, two types of threshing machines are analysed:
- The tangential threshing machine in the Claas Lexion 750 self-propelled combine harvester;
- The axial threshing device in the John Deere S 670i self-propelled combine.

The parameters of the threshing apparatus, from the two types of self-propelled combines for harvesting cereals, are centralised in Table 3.

Table 3. Parameters of the threshing apparatuses studied

Parameter	UM	Self-propelled combine	
		Claas Lexion 750	John Deere S 670i
Beater / rotor diameter	mm	600	762
Beater width, Rotor length	mm	1420	3124
Beater speed	rot./min	450-1050	210-1000
Beater angular speed	rad./sec	46-108	22-105
Centrifugal acceleration	m/s^2	635-3498	193-4410
Thresher cinematic index	K	64-357	20-448
Counter-beater wrapping angle	degrees	142	124
Counter-beater area	m^2	1.45	1.54
Thresher feeding flow	kg/sec	22	21
Hourly combine productivity	t/h	48	42

CONCLUSIONS

The study on the dynamics of the threshing machines of the grain harvesters was carried out on a number of two combines: one with tangential flow and one with axial flow.
From the analysis of the technical characteristics of the threshing apparatuses of these self-propelled combines, the following conclusions can be drawn:
- The Claas Lexion 750 self-propelled combine is equipped with a tangential flow thresher with APS pre-pressing accelerator and Multicrop type counter-balancer.
The double threshing surface - a pre-separation grate and a Multicrop counter-beater - make the thresher highly productive, reducing energy and fuel consumption.
- The John Deere S 670i self-propelled combine is equipped with an axial thresher with a single rotor (bullet rotor).
The separation cylinder on the outside of the rotor is divided into three concentric sectors of different diameters.
Due to this special construction, the material does not agglomerate inside the cylinder and there is no risk of clogging the device with material.
Due to the expansion movement of the material, it is energetically loosened and, therefore, harvesting is possible even at higher humidity.

Therefore, it can be seen that grain harvesters tend to use double threshers with tangential flow and threshers with axial flow, due to the following advantages: both threshing and the separation of grain from straw are achieved; the length of the coulter and the vibrations are reduced since the shaker is no longer used for separation; and the percentage of unthreshed ears is reduced because the material is driven in a roto-translational movement inside the threshing machine.

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THE IMPACT OF MONITORING THE STATE OF VEGETATION OF LAVENDER CROPS USING ANALYSIS DRONES

Cristinel FERTU[1], Zanet VITALII[1], Daniela Lavinia BALASAN[1], Silvius STANCIU[1], Daniela NENCIU (COADA)[2], Raluca-Beatrice COSTEA (VOINEA)[1]

[1]"Dunarea de Jos" University of Galati, 47 Domneasca Street, Galati, Romania
[2]Iasi University of Life Sciences "Ion Ionescu de la Brad", 3 Mihail Sadoveanu Alley, Iasi, Romania

Corresponding author email: cristi_fertu@yahoo.com

Abstract

Agriculture evolves from day to day, so that, among the traditional crops, whole fields of lavender have appeared. Apart from its decorative uses, lavender has many other uses, both in the cosmetic and food industries. Lavender growing can become a profitable business for farmers, entrepreneurs and gardeners alike. Lavender is often grown for the benefits of the products it produces. Lavender is a perennial plant that is rarely attacked by pests. As it is considered a niche business, lavender is often grown on small areas. Analysis of the state of vegetation and condition of agricultural crops is done using multispectral drone cameras. These combine data from several separate sensors, so that it is possible to quickly analyse the evolution of the plants, the presence of weeds and insects, the lack of water, etc. In this article we will highlight the impact of using a drone analysis for the purpose of monitoring the state of vegetation as well as the opportunities in finding solutions to control pests and other factors on a field cultivated with lavender in Campia Romana.

***Key words**: unmanned aerial vehicle (UAV), precision agriculture, normalized difference vegetation index (NDVI), green normalized difference vegetation index (GNDVI).*

INTRODUCTION

Agricultural drones give farmers access to in-depth data analysis and farm work planning, as well as new tools for more efficient resource management to reduce manual labour. For farmers, as well as other organisations and institutions involved in crop cultivation and research, drone technology can help them exceed their crop establishment goals and achieve more with fewer resources. Drones can provide high-quality, high-resolution images even on cloudy days (Manfreda et al., 2018). The availability of the drones to be used under different conditions and the speed of transmission are other advantages to be mentioned in literature (Radoglou-Grammatikis et al., 2020). Compared to airplanes, drones are much cheaper and easier to install and maintain (Tsouros et al., 2019). There are several application areas for drones in agriculture. Drones can be integrated with new technologies, capabilities of computing and on-board sensors to support crop management (mapping, monitoring, irrigation, crop diagnostics, etc.) (Huang et al., 2021). Disaster risk reduction, early warning systems, nature and forest conservation, are again other areas that analysis drones can achieve. Drones can be used not only for surveillance, estimation and detection based on sensor data, but also for crop irrigation and weed, pest and disease management. This means that drones can apply precise amounts of water and pesticides based on the information obtained (Rejeb, 2022). The benefits of drones in agriculture are summarised in Figure 1.

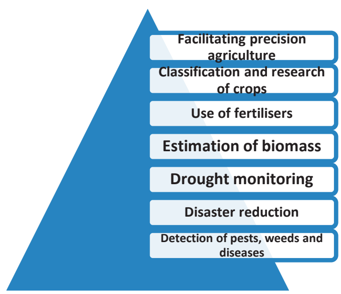

Figure 1. Benefits of drones in agriculture
(Source: Authors' contribution)

Precision agriculture offers opportunities to improve crop productivity and farm profitability through better management of farm inputs (Larson & Robert, 1991; Zhang, Wang & Wang,

2002) and improved environmental quality (Mulla, 1993; Mulla et al., 1996; Tian, 2002). Many farmers strive to apply the latest technology in practice to maximize profits (Seelan et al., 2003), but costs, delivery and information retrieval (especially with remote sensing) may be a barrier to their use in agriculture (Stafford, 2000). Compared to other methods, the use of drones implies much lower cost advantages and significantly better results. Images captured by agricultural analysis drones (UAV - unmanned aerial vehicle) usually have higher radiometric uniformity than aircraft or satellite images due to the lower acquisition altitude (Lelong et al., 2008). However, UAV systems also inherit their own image quality problems. For example, the light weight of many UAV systems means that the camera position is not stable, resulting in different spatial resolutions and/or different viewing angles for different images along the same aircraft (Lelong et al., 2008). The low altitude of these craft can also lead to large geometric distortions (Lelong et al., 2008; Xiang and Tian, 2011). In addition, because of the low altitude, the number of UAV images in each field is also large. There are also technical problems with images captured by UAV-mounted cameras, such as blurring caused by forward motion of the image. Oversampling is usually used to compensate for this but will inevitably lead to increased data volume (Aber et al., 2010).
This improves the data collection process and provides insight into plant health and vegetation management. Given the large amount of images acquired, image decorrelation is a necessary pre-processing step.

MATERIALS AND METHODS

The DJI P4 RTK multispectral cluster drone will be used for research in this article confirm Figure 2.

Figure 2. DJI P4 RTK drone
(Source: Authors' contribution)

Although manual geometric correction has been successful (Hardin et al., 2007; Vericat et al., 2008), it is not suitable for applications in AP (Precision Agriculture) where a larger area of agricultural crops needs to be monitored. There is a possibility for optimizing the turnaround time (i.e. the time it takes to process the final product and get it to the user) which is a major concern for these devices, as hundreds of images are often captured and require a quick response from the manufacturer. As a result, automated processing often dominates UAV data processing. Automated or semi-automated (block file) photogrammetry systems can successfully cope with the roll, pitch, and yaw variations common in UAVs (Laliberte, 2011; Xiang, 2011). However, due to the small scan strip area and unstable platform, geometry and orthorectification are also required before combining images. The methods developed to solve these problems include: manual georeferencing using GCPs (ground control points) collected in the field, photo matching, and automatic georeferencing using navigation data and camera lens distortion models (Xiang, 2011). Agricultural imaging obtained is easier and more efficient than ever with a built-in stabilised imaging system that captures complete data sets immediately. The information is collected by an RGB camera and a series of five-camera multispectral cameras as shown in Figure 3, covering the blue, green, red, red and near-infrared bands. All these cameras are 2MP in size and have a global shutter on a 3-axis stabilized gimbal. An integrated solar spectrum sensor on top of the drone captures solar radiation, maximising the accuracy and consistency of data collection at different times of the day.

Figure 3. DJI P4 multispectral camera
(Source: Authors' contribution)

All users who own a drone that weighs more than 250g or is equipped with a camera and microphone must register with the Romanian Civil Aviation Authority before obtaining a flight permit. Using the DJI GS PRO app (the main iOS app for DJI flight planning) compatible only with iPhone devices (tablets), flight missions are set up on the investigated plots as shown in Figure 4.

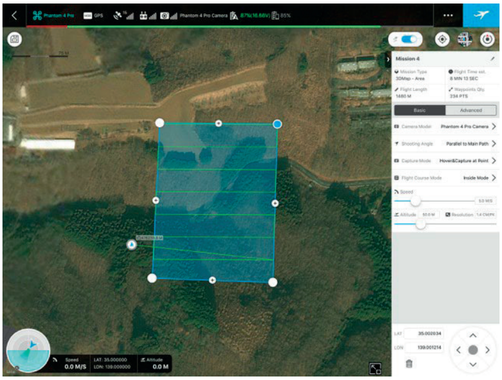

Figure 4. DJI GS PRO application
(Source: Authors' contribution)

After the drone flight mission is set up over the lavender fields in the Baragan Plain, images of the vegetation condition of the studied crop, specifically the lavender crop, will be collected. After several flight missions, these images will be analysed and, of course, we will try to interpret these images through the colour spectrum, both in terms of fertilisation and the use of plant protection products. The images obtained from the flight mission over a herb crop plot with all 6 multispectral cameras can be seen in Figure 5 as follows:

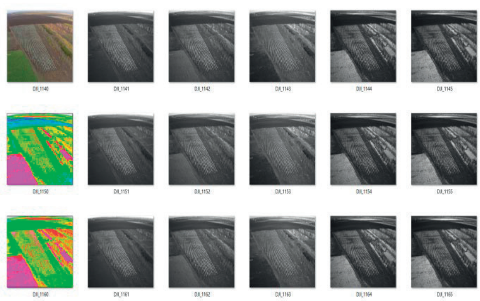

Figure 5. Images obtained with the DJI 4 RTK
(Source: Authors' contribution)

PIX4Dfields is advanced agricultural mapping software for plant analysis and digital agriculture. Therefore, after obtaining high-resolution images in natural or multispectral colour, the PIX4Dfields application provides easy access to a wide range of tools and functions for creating orthomosaics, vegetation indices and annotations. Performing this statistical analysis as shown in Figure 6, is shown by inserting all collected images. Regardless of the type of drone or camera, PIX4Dfields requires a careful analysis of the flight plan parameters in order to obtain and process an appropriate data set.

Figure 6. Merging the collected images and obtaining NDVI index maps (Source: Authors' contribution)

NDVI (Normalised Difference Vegetation Index) quantifies vegetation by measuring the difference between near infrared (which vegetation reflects strongly) and red light (which vegetation absorbs).

RESULTS AND DISCUSSIONS

Two types of crop mapping maps are produced using drones. RGB and NDVI (Normalized Difference Vegetation Index) maps showing the amount of infrared light reflected in an area, providing a panoramic view and allowing crop monitoring - an indicator of crop health. NDVI is a dimensionless index that represents the difference between visible and near-infrared reflectance of vegetation cover, it can be used to estimate the green density of upland areas (Weier and Herring, 2000). NDVI is calculated as the difference between near infrared (NIR) and red (RED) divided by the sum.

$NDVI_i=(NIR-\text{ROȘU})/(NIR+\text{ROȘU})$,

$NDVI_i$ represents the smoothed NDVI (NDVI) observed at time step i and their ratio gives a measure of photosynthetic activity in values between -1 and 1. Low NDVI values indicate moisture-stressed vegetation and higher values indicate higher density of green vegetation. According to the interpretation results using the Pix4Dfields application together with the QGIS application, we obtained an area map of the analysed plots, so as shown in Figures 7 and 8.

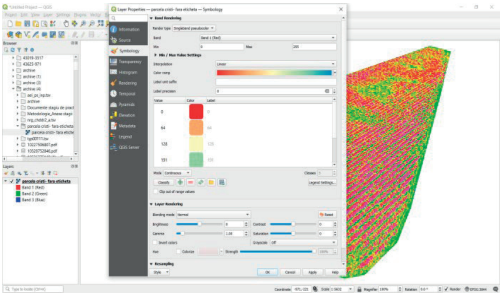

Figure 7. Obtaining histograms and spectral bands (Source: Authors' contribution)

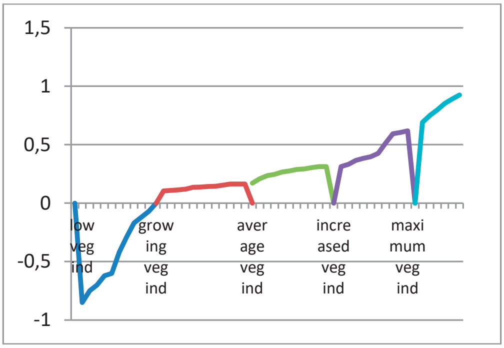

Figure 8. Results of vegetation indicators of the studied crop (Source: Authors' contribution)

From the zonation map we can see that the values obtained range from -1 to +1. where -1 indicates an extremely low degree of vegetation and +1 indicates a high degree of vegetation. The processing software separates these values by colour to better distinguish between diseased and healthy vegetation in a given area. The normalised vegetation index (NDVI) is
the oldest, best known and most widely used vegetation index. The spectral range with the highest degree of absorption and reflection associated with chlorophyll activity is used for the calculation required in our crop. For green vegetation, the most common range is between 0.2 and 0.8. Almost 75% (74.90%) of the crops surveyed were observed to have vegetation conditions at medium to high levels. These calculations automatically lead, in the case of foliar products and plant protection products, to a decrease in the quantity used during the harvest of herbs. The savings are significant in terms of fuel used in conventional machinery and, of course, in terms of marketed inputs compared to conventional methods that allow for crop control and farm operation expenses, will be around 25%.

CONCLUSIONS

The introduction of drones in agriculture will allow this branch of economy to reach a new level in terms of the quality of the harvests obtained in strict accordance with the Common Agricultural Policies (CAP) in order for farms to be competitive and efficient on national and international markets. Monitoring plant health using drones to make correct 'phytosanitary' decisions based on the number of affected plants (weeks before emergence) leads to accurate estimation of infection rates and proper fertiliser application. The same aspect is definitely achievable in the case of monitoring the vegetative state of plants for the use of plant protection product treatments in collaboration with a careful monitoring and evaluation of the damage done after weather events due to the climate change. Information-based technologies need to be developed and promoted for the vast majority of agricultural crops and of course at affordable prices. In this way, agricultural drones ensure efficient surveillance of agricultural crops, simplify the use of new technologies, so that they become a tool for collecting data on established crops, data that will be of real use to the entire farming community.

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EVALUATION OF STICKINESS OF PLANTS PROTECTION PRODUCTS IN THE LABORATORY CONDITIONS

Donyo GANCHEV, Atanas IVANOV

Agricultural University of Plovdiv, 12 Mendellev Blvd, Plovdiv, Bulgaria

Corresponding author email: donyo@abv.bg

Abstract

The stickiness of the plant protection products, means their ability to sustain washing out from sprayed plant surfaces from rain, is one of the most critical and important attributes, especially for protective action non-systemic pesticides. Lack or insufficient stickiness will cause a greatly dropping in effectiveness, especially during rainy weather (seasons), which on the other side, will prove favourable conditions for spreading pests on plants. Therefore, selecting plant protection products with good rain retention ability is critical for achieving a satisfactory level of effectiveness in the case of treatments in such weather conditions. This of course, invokes the need to evaluate of this property, which is the object of the given research. Plant protection products were tested alone and in combinations with sticky and wetness agents for evaluation their stickiness ability. The results shows that stickiness ability can vary greatly for different plant protection products, but addition of sticky agent to the pesticide solution can improve it significantly in most of the cases. Hoverer there was some exceptions.

***Key words**: pesticides, stickiness, rain resistance, effectiveness.*

INTRODUCTION

Stickiness of the plant protection products which means their ability to sustain of washing out from sprayed plant surfaces from rain is one of most critical and important attribute especially for protective action plant protection products. Lack or insufficient stickiness cause greatly dropping of the effectiveness especially during rainy weather (seasons) which from the other side will prove favourable conditions for spreading pests on plants. Therefore, selecting the plant protection product with the good rain retention ability. This of course invoke the need of evaluation of this property which is the object of the given research and paper respectively (Trevisan et al., 1993; Gossen et al., 2008). The washing out of the pesticides from treated plant surfaces not only decrease their effectiveness but can also cause significant pollution of the environment, especially soils, underground and surface waters (Hüskes & Levsen, 1997; Bruce et al., 1975; Ahmed et al., 1998).

Using the pesticides with goods stickiness to the plant surfaces or adding the sticker adjuvant onto spayed solutions is also recommended as a strategy for combating the resistance towards the pesticides (Beresford et al., 2005). The stickiness of the pesticides also is affected from addition of different adjuvant especially wetting and sticking agents to the spaying solutions. Typically rain retention of the pesticides is evaluated with spectrophotometry which provide from one side very credible results but from the other - is completely impossible to be used from the regular agronomists and farmers, plus the price and time for conducting suck king of evaluations (Decaro et al., 2016). Especially that there is a differences in retention and rainfastening properties between commercial stickers of the same chemical type (Taylor & Matthews, 1986; Gaskin & Steele, 2009). Large differences in roughness, in the amount and composition of surface waxes and in the retention and rain fastness of mancozeb were found among species in the study was made of the influence of the upper leaf surface characteristics on the retention and rain fastness of the contact fungicide mancozeb with and without tank-mix adjuvant (RSO 5 and RSO 60) on apple seedlings, bean seedlings and kohlrabi plants. Rain fastness correlated strongly or very strongly with the amount of C28 alcohol and C33 alkane. The addition of a more hydrophobic (RSO 5) or a more hydrophilic (RSO 60) adjuvant to the spray solution influenced retention and rain fastness, and also altered the correlation coefficients (Hunsche et al., 2006).

The formulation of the plant protection products i.e. addition of the adjuvant into them actually greatly affect rain retention of the pesticides (Lopez & Hua, 1970). Was established that pesticides formulated as wettable powders were retained by a greater amount than those formulated as emulsifiable concentrates (Cooper & Hall, 1993), but the increase of surfactants in the pesticide sprays (decreasing the surface tension) lead to the reduction of the rain retention (Prado et al., 2016). However, this statement is controversial because other studies reveal the opposite (Basu et al., 2002). However, the structure of the leaf surface also can affect the stickiness of the pesticides. Plant species with crystalline epicuticular waxes like pea or wheat retained much less spray solution than the other species, which are characterized by a smooth cuticular surface (De Ruiter et al., 1990). In the similar study, the adhesion of the spreader-Sticker adjuvants was evaluated by using PARAFILM M, Bemis NA, Neenah, WI pieces weighed before and after the test i.e. dropping onto them the pesticide solution. The pieces were dipped into a beaker containing 500 ml de-ionized (DI) water either 100 times, 200 times, or 300 times at an approximate dipping rate of two dips/s (Meredith et al., 2014)

MATERIALS AND METHODS

A standard glass slides for microscope observations were used for the trials. Tested plant protection product solutions were dropped onto slides (0.5 ml per slide). After drying of the solutions, slides were placed into 400 ml plastic cups filled with water, at 45° angle. The cups were covered with scratch transparent kitchen folio and were placed on shelf at 24-25°C temperature and no direct sunshine for 14 days. After this period, the slides were pulled out from the cups and visual observations and measurement for degree of retention were conducted with millimetre paper. This is a typical method for evaluation of the sticky abilities of the plant protection products by using so called "tracers" (Allagui et al., 2018). Were conducted tests with several plant protection products:

1. **Delaro 325 SC** - fungicide on the base of prothioconazole - 175 g/L and trifloxystrobin - 150 g/L at 0.08% concentration;
2. **Forester EW** - insecticide on the base of cypermethrin - 100 g/l at 0.2% concentration;
3. **Indaziflam 500 SC** - herbicide on the base of Indaziflam - 450 g/l at 0.02% concentration;
4. **Qilt Excel SE** - fungicide on the base of azoxystrobin - 135 g/l and propiconazole - 117 g/l at 0.1% concentration;
5. **Capito SC** - insecticide on the base of indoxacarb - 75g/l and abamectin - 18 g/l at 0.1% concentration;
6. **Daxur SC** - fungicide on the base of mefentrifluconazole - 100 g/l and kresoxim methyl - 150 g /l at 0.1% concentration;
7. **Fluxapiprolin 20 SC** - fungicide on the base of fluxapiprolin - 200 g/l at 0.1% concentration;
8. **Traciafin Plus EC** - fungicide on the base of prothioconazole - 250 g/l at 0.08% concentration;
9. **Enevrvin SC** - fungicide on the base of ametoctradin - 200 g/l at 0.15% concentration;
10. **Mikal Flash WG** - fungicide on the base of fosetyl aluminium - 500 g/kg and folpet 250 g/kg at 0.3% concentration;
11. **Cabrio Top WG** - fungicide on the base of methiram - 550 g/kg and pyraclostrobin - 50 g/kg at 0.2% concentration;
12. **Delan Pro SC** - fungicide on the base of dithianon - 125 g/l at 0.05% concentration;
13. **Dithane M-45 WP** - fungicide on the base of mancozeb - 750 g/kg at 0.25% concentration;
14. **Funguran OH 50 WP** - fungicide on the base of Copper (II) hydroxide - 770 g/kg at 0.3% concentration;
15. **Triomax WP** - fungicide on the base of cymoxanil - 40 g/kg, copper oxychloride - 290 g/kg and mancozeb - 120 g/kg at 0.25% concentration;
16. **Manex C-8 WP** - fungicide on the base of cymoxanil - 80 g/kg and mancozeb - 600 g/kg at 0.15% concentration;
17. **Medody Compact 49 WG** - fungicide on the base of iprovalicarb - 84 g/kg and copper oxychloride - 406 g/kg at 0.15% concentration;
18. **Bordomix 20 WP** - fungicide on the base of bordeaux mix - 200 g/kg at 0.5% concentration;
19. **Kumulus DF WG** - fungicide on the base of sulfur - 800 g/kg at 0.3% concentration;
20. **Thiozole 80 WP** - fungicide on the base of sulfur - 800 g/kg at 0.3% concentration;
21. **Thiovit Jet 80 WG** - fungicide on the base of sulfur - 800 g/kg at 0.3% concentration;
22. **Curzate 60 WG** - fungicide on the base of cymoxanil - 600 g/kg at 0.25% concentration;

23. **Cuprozin Super M WP** - fungicide on the base of mancozeb - 200 g/kg and copper oxychloride -500 g/kg at 0.4% and 0.2% concentrations;
24. **Champion WP** - fungicide on the base of Copper (II) hydroxide - 770 g/kg at 0.3%.

The stickiness of the plant protection products were evaluated alone and with addition of sticky agents to the solutions: Elect 90 EC© on the base of paraffin oil at 0.2 % concentration and Strong Oil© on the base of plant triglyceride oil at 0.5% concentration, and with addition of wetting agents to the solutions: Silwet L-77© on the base of organosilicone surfactant at 0.1% concentration and 2.Spur© on the base of organosilicone surfactant at 0.1% concentration.

RESULTS AND DISCUSSIONS

In the Table 1 are presented results from conducted test for stickiness of plant protection products applied alone without adjuvants.

Table.1 Stickiness of tested plant protection products used without adjuvants

Plant Protection Product	Percent Retention
Mikal Flash - 0.3%	0
Cabrio Top - 0.2%	100
Delan Pro - 0.05%	100
Dithane M-45 - 0.25%	85
Funguran OH 50 WP - 0.3%	100
Triomax - 0.25%	100
Manex C-8 - 0.15%	60
Medody Compact 49 WG - 0.15%	80
Bordomix 20 WP - 0.5%	100
Kumulus DF - 0.3%	100
Thiozole 80 WP- 0.3%	15
Thiovit Jet 80 WG - 0.3%	90
Curzate 60 WG - 0.25%	95
Cuprozin Super M - 0.4%	98
Cuprozin Super M - 0.2%	100
Champion - 0.3%	100
Delaro 325 SC - 0.08%	2
Forester - 0.2%	10
Indaziflam 500 SC - 0.02%	10
Qilt Excel - 0.1%	0
Kapito - 0.1%	0
Daxur - 0.1%	100
Fluxapiprolin 20 SC - 0.1%	0
Traciafin Plus - 0.08%	2
Enevrvin - 0.15%	90
Pasta Caffaro - 0.03%	60
Electis Cobre - 0.3%	95
Micrithiol Dispers Sulfur - 0.6%	95
Arrone - 0.73%	0

From table above is clear that some of the plant protection products as: Cabrio Top, Delan Pro, Funguran OH 50 WP, Triomax and others have a 100% retention (stickiness) and their application do not require any addition of sticky agents. However other plant protection products as: Mikal Flash, Delaro 325 SC, Qilt Excel, Arrone and others actually were completely washed out in the conducted trials**.**

Addition of sticky agents to the pesticide solutions improve significantly their retention ability (Figures 1 and 2).

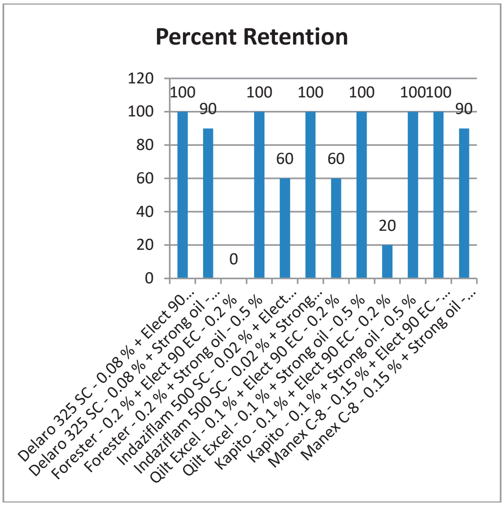

Figure 1. Stickiness of tested plant protection products with addition of sticky agents

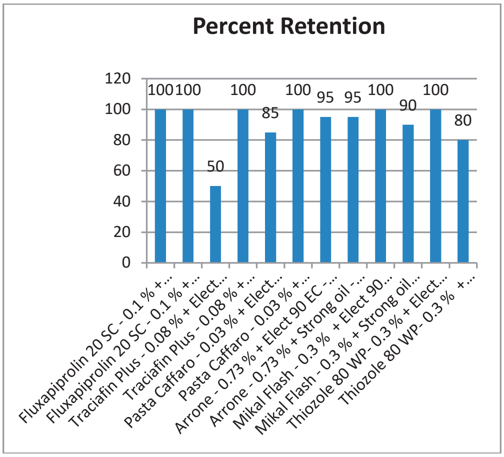

Figure 2. Stickiness of tested plant protection products with addition of sticky agents

However, in some products were received differences according used two different sticky agents: Elect 90 EC on the base of mineral oil and Strong Oil on the base of plant derived oil. Forester mixed with Elect 90 EC was completely washed out while with Strong Oil has 100% retention. Similar results were received and with Indaziflam 500 SC, Qilt Excel, Kapito and Traciafin Plus. Tests with Thiozole 80 WP shows the opposite – stickiness with Strong Oil was better than with Elect 90 EC. Tests with Delan Pro shows that addition of sticky agents actually decrease the retention of the product. Delan Pro used alone have 100% retention, while with addition of Elect 90 EC, only 10% and with addition of Strong Oil - 60%. Similar results was received with Triomax which alone also have 100 % stickiness. With addition of Strong oil, this property drop to 60%. However addition of Elect 90 EC maintain 100% stickiness. The same situation was received with other products on the base sulphur Kumulus DF and Thiozole 80 WP where the addition of Strong Oil drop the retention with 20%.

In the Table 2 are presented results from conducted test for stickiness of plant protection products applied together with organosilicone surfactant (Silwet L-77 and Spur) in 0.1% (v/v) concentration for the surfactant:

Table 2. Stickiness of tested plant protection products with adding organosilicone sufactant to the solutions

Plant Protection Product	Percent Retention
Delaro 325 SC - 0.08% +surfactant- 0.1%	1
Forester - 0.2% + surfactant - 0.1%	0
Indaziflam 500 SC - 0.02% + surfactant - 0.1%	0
Qilt Excel - 0.1% + surfactant- 0.1%	0
Kapito - 0.1% + surfactant - 0.1%	0
Daxur - 0.1% + surfactant - 0.1%	0
Fluxapiprolin 20 SC - 0.1% + surfactant- 0.1%	0
Traciafin Plus - 0.08%+ surfactant - 0.1%	0
Enevrvin - 0.15% + surfactant - 0.1%	10
Pasta Caffaro - 0.03 % + surfactant - 0.1%	60
Electis Cobre - 0.3 % +surfactant - 0.1%	75
Micrithiol Dispers Sulfur - 0.6% + surfactant - 0.1%	55
Arrone - 0.73% + surfactant - 0.1%	0
Mikal Flash - 0.3% + surfactant- 0.1%	0
Cabrio Top - 0.2% + surfactant- 0.1%	40
Delan Pro - 0.05% + surfactant - 0.1%	0
Dithane M-45 - 0.25% + surfactant- 0.1%	100
Funguran OH 50 WP - 0.3% + surfactant - 0.1%	100
Triomax - 0.25% + surfactant - 0.1%	100
Manex C-8 - 0.15% + surfactant - 0.1%	100
Medody Compact 49 WG - 0.15% + surfactant - 0.1%	100
Bordomix 20 WP - 0.5% + surfactant - 0.1%	100
Kumulus DF - 0.3% +surfactant - 0.1%	20
Thiozole 80 WP- 0.3% + surfactant - 0.1%	3
Thiovit Jet 80 WG - 0.3% +surfactant - 0.1%	80
Curzate 60 WG - 0.25% + surfactant - 0.1%	100
Cuprozin Super M - 0.4% + surfactant - 0.1%	90
Cuprozin Super M - 0.2% + surfactant - 0.1%	100
Champion - 0.3% + surfactant - 0.1%	100

From the table above is can be see that addition of surfactant to the pesticides solutions in the most cases decrease the stickiness to the 0%. However, in some of the products as: Champion, Cuprozin Super M, Dithane, Manex C-8 and others, the retention is not affected. From the three sulfur based plant protection products: Kumulus DF, Thiozole 80 WP and Thiovit Jet 80 WG, in the last one addition of surfactant drop the retention only with 20 %. Solutions of Kumulus DF and Thiozole 80 WP with surfactant shows stickiness 20 and 3%.

Addition of sticky agents (Elect 90 EC and Strong Oil) to the solutions of plant protection products combined with surfactant restore their retention to 90-100%, however only towards some of products as: Enevrvin, Pasta Caffaro and Micrithiol Dispers Sulfur. Towards other products as: Forester, Indaziflam 500 SC, Daxur, Fluxapiprolin 20 SC, Electis Cobre and Cabrio Top such action was observed towards Strong Oil but not towards Elect 90 EC. In the solutions of Thiovit Jet 80 WG was the opposite. Towards other plant protection products as: Delaro 325 SC, Qilt Excel, Kapito, Traciafin Plus, Arrone, Mikal Flash, Delan Pro, Kumulus DF and Thiozole 80 WP addition of both sticky agents (Elect 90 EC and Strong Oil) to the solutions of plant protection products combined with surfactant do not improve their retention ability.

CONCLUSIONS

From conducted trials can be concluded that stickiness of the plant protection products greatly variable and addition of sticky agents do not improve it in the all cases. Different products can express different sticky ability when they are mixed with different sticky agents or combination of sticky agents and surfactants. However, there were no differences in the action between two organosilicone surfactants used in the tests. Some of tested plant protection products as: Dithane M-45, Funguran OH 50 WP, Medody Compact 49 WG, Cuprozin Super M shows good levels of retention alone and no matter with what kind adjuvant are mixed. In some products as Champion, Cuprozin Super M, Curzate 60 WG and Bordomix 20 WP, this retention percent was 100%. The tests reveal that always the good stickiness of the plant protection products had to be tested alone and with combinations of selected adjuvants for best effectiveness and performance before use.

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MATHEMATICAL EVALUATION OF TECHNOLOGICAL APPROACHES FOR CORIANDER PRODUCTION

Velika KUNEVA[1], Vanya DELIBALTOVA[1], Svetlana MANHART[1], Manol DALLEV[1], Ivan MITKOV[1], Dimitar RAZPOPOV[1], Galya HRISTOVA[2]

[1]Agriculture University of Plovdiv, 12 Mendeleev Blvd, Plovdiv, Bulgaria
[2]Trakia University, Stud. Grad., Stara Zagora, Bulgaria

Corresponding author email: manol_dallev@abv.bg

Abstract

Recently, the cultivation of essential oil and aromatic crops is becoming more and more popular. A typical representative of such a culture is coriander. The article presents real data from the coriander production process, using two different technological approaches. By using a mathematical, dispersion analysis and T-test, the technology is evaluated. The obtained results are adequate and easy to interpret, they reflect the entire process, but they are valid only under the specific conditions. However, it can be clearly emphasized that sowing term affects yield when cultivate coriander.

Key words: *coriander, technology for production, sowing term, dispersion analysis.*

INTRODUCTION

Coriander is one of the main essential oil crops cultivated in Bulgaria. They are mainly used for fruits (seeds) with an annual production of about 20,000 t, which are mainly exported to Sri Lanka, Indonesia, Singapore, Malaysia and Great Britain. Under the current market conditions, one of the main ways to increase yield and improve the quality of coriander production is the development and application of effective technologies for its cultivation. Sowing data is one of the most important agronomic factors, playing an important role in the expression of plant genetic endowments (Diederichsen, 1996). This key agronomic factor is determined depending on the direction of production and the specific soil and climatic conditions of the area (Ghobadi and Ghobadi, 2010).

The time of sowing has a significant impact on the seed yield of coriander and the determination of the optimum time determines the productivity. In India, the highest seed yield was obtained in coriander sown from mid to late October, but the highest essential oil content was obtained when sown from early to mid-October (Gujar et al., 2005; Khah, 2009). However, sowing beyond the optimum time seriously reduced yield as well as essential oil content.

A study by Sudeep et al. (2005) showed that October sowing compared to December sowing is more suitable for coriander because higher values of structural elements and seed yield are obtained. Other authors have identified the climatic conditions of the year as the determinant in terms of coriander seed yield and essential oil content. The year with the highest amount of rainfall produces the highest seed yield but lower essential oil content (Kuri et al., 2015).

Zheljazkov et al. (2008) conducted a study in Canada with two coriander cultivars (Jantar, Alekseevski) to determine the effect of two sowing data (May 24 and June 8) on seed and essential oil yield. The results showed that the highest seed and essential oil yields for both cultivars were obtained when sown on 24 May, with insignificant differences between Jantar and Alekseevski varieties.

According to Luayza et al. (1996), in Argentina, autumn-winter sowing of coriander is optimal to achieve the highest yield. Another study conducted in Italy (Carruba et al., 2006) found that December sowing provided over 90% higher yield compared to January, February, March and April sowings. Sharangi and A. Roychowdhury (2014) found that late sowing reduces plant height, number of tillers per plant, while sowing at optimum times results in increased yield and productivity elements. Bhadkariya et al. (2007) investigated the effect of several sowing dates (October 25, November 5, November 25 and December 5) on structural elements of yield, yield and seed quality. Maximum values of all the parameters studied were obtained when coriander was sown in early November (5 November). Some authors recommend sowing no later than 30 March (Zareie et al., 2012). Sowing coriander between 30 March and 29 April significantly reduces seed yield (Moosavi, 2012). A study in Poland by Nowak, & Szempliński (2014), showed that early sowing increased seed yield.

In a study conducted in the Karnobat region of southeastern Bulgaria, they investigated the effect of sowing time on coriander productivity and found that sowing in April provided 30-35% lower seed yields and essential oil content compared to February and March sowing (Gramatikov et al., 2005).

For Dobrudzha conditions, Tonev and Gramatikov (2008) recommend sowing coriander in March, while in the Karnobat region the highest seed yield is obtained when sowing in February (Gramatikov et al., 2005).

MATERIALS AND METHODS

Good precursors for coriander with crops that release the area early. Most suitable are winter cereals and grain legumes, suitable are vegetable crops and early maize hybrids, and less suitable are sunflowers, cotton and tobacco. Unsuitable for coriander with sorghum and sugar beet. On the same field to return no earlier than 2-3 years. Coriander is a good precursor for all winter-cereals and other crops sown in autumn.

Annually on the coriander farm is found after the wheat precursor.

Company "Agro Impulse" Ltd. Voyvodinovo, grows coriander, variety "Local small-fruited". This variety was created in the conditions of Bulgaria and is distinguished by thin, upright, light green stems, about 65-70 cm tall. The flowers are clustered in sparse and small umbels of 3-6 rays. Fruits are brown to yellow, small, 1.5-2.0 mm in diameter and 5-6 g per 1000 fruits. Its ripeness at full maturity at harvest is not high. The variety is relatively early maturing, with a growing period of 100-105 days and an essential oil content of 1.25%.

During the period 2020-2022 in the village of. Voyvodinovo, Maritsa municipality, three annual field trials were conducted using the fractional plot method in 4 replications with a harvest plot size of 15 m^2. Precursor - wheat. The tillage depending on the sowing period included:

In autumn sowing of coriander

After wheat harvest - July-August, summer ploughing is carried out at 18-20 cm. In the period August-September - disking at 13-15 cm and immediately before sowing - September-October - cultivation at 6-8 cm.

For spring sowing of coriander

After harvesting the precursor, July-August deep ploughing at 22-25 cm is carried out. During the period October-November - disking at 13-15 cm, at the first opportunity to enter the block in spring cultivate at 8-10 cm (early February) and immediately before sowing - cultivation with harrowing at 6-8 cm (late February - early March).

Coriander is sown at a seed rate of 1.5-2.0 kg/da, at a spacing of 12-15 cm, at a depth of 3-4 cm. Mandatory post-sowing rainfall.

Application of nitrogen fertiliser at a rate of 5-6 kg/da N is carried out after germination. In the case of coriander sown in autumn - February-March, and in the case of those with spring - March-April.

Praxim herbicide (500 g/l Metobromuron) - 220 ml/da applied in March-April was used for weed control.

At the end of the budding phase of coriander plants - May foliar fertilization with Masterblad was applied at a rate of 250 ml/da.

Harvesting was carried out in July with a single-phase combine harvester at the full maturity stage with recorded seed moisture not higher than 9%.

For 100 kg of fruit the coriander extracts 3-3.5 kg N, 3.5 kg K_20, 1.1 kg P_20_5 fertilization is carried out with 1-3 t/da manure. Depending on the soil fertiliser content, mineral fertiliser is applied - 10-15 kg N/day, 8-10 kg/day P_2O_5, 6-8 kg/day K_2O on lightly fertilised soils and 8-10 kg N/day, 6-8 kg/day P_2O_5, 4-6 kg/day K_2O on medium fertilised soils. Manure, phosphorus and potassium fertilisers are applied with ploughing and nitrogen fertilisers just before sowing.

The results of the soil analysis made by the Laboratory Complex at AU - Plovdiv, after harvesting the precursor on the farm are presented in Table 1.

The data showed that the total nitrogen content before sowing of coriander was 9.71 mg/kg soil in the 0-20 cm layer and 9.20 mg/kg soil in the 20-40 cm layer. According to Gorbanov St. et al. (1990), soils are poorly stocked and the application of nitrogen fertilizers is necessary for the optimal development of coriander.

The uptake phosphorus content in the 0-20 cm layer was 19.00 mg/100 g soil and in the 20-40 cm layer was 24.68 mg/100 g soil. According to the methodologies of Truog, Olsen and Egner Reim (Gorbanov St. et al., 1990) the stock is good.

The uptake potassium content determined in the extract with 2N HCI was good 25.40 and 31.79 mg/100 g soil at 0-20 cm and 20-40 cm, respectively.

Due to the good soil phosphorus and potassium availability, no application of these trace elements is required.

The economically important diseases of coriander are bacteriosis, cercosporosis, ramulariosis, leaf spot and bacterial blackening of the fruit, and of the pests, coriander seed weevil, coriander aphid and meadow weevil. The control is carried out with registered fungicides and insecticides, as well as with agrotechnical means - observing crop rotation, selecting a suitable precursor, bulking and ploughing of crop residues, and sowing a weed-free area.

RESULTS AND DISCUSSIONS

Technological maps have been developed from the presented technological solutions for growing coriander (Photo 1).

Photo 1. Trial fields of coriander

Table 1. Technological map for spring sowing of coriander, after predecessor wheat

№	Technological operations	Agrotechnical requirements	Volume of work - V p , (ha, t, t.km, pcs.)	Agrotechnical period	Planned timeline from .... to	Number of working days - Dr	Duration of the working day - Td	Number of mechanics - nmex	Number of support workers - npr	Hourly productivity - Wh	Daily productivity - Wдн	Productivity for the planned period - W pl
1	Ploughing	20-25 cm	100 ha	VII-VIII	1-10.VIII	11	8	1	0	1.18	9.4	103.4
2	Discus	13-15 cm	100 ha	X-XI	1-3.X	3	8	1	0	0.5	38.4	115.2
3	Cultivation	8-10 cm	100 ha	II	1-3.II	3	8	1	0	5.04	40.3	120.9
4	Cultivation + harrowing	6-8 cm	100 ha	II	26-28.II	3	8	1	0	5.04	40.3	120.9
5	Sowing	15-20 kg/ha	2 t	II-III	1-3.III	3	8	1	0	4.69	37.5	112.5
6	Fortified		100 ha	II-III	1-3.III	3	8	1	0	6.75	54	162
7	Fertilization	150 kg/ha (N)	15 t	III-IV	1.IV	1	8	1	1	24.38	195	195
8	Spraying with herbicide (Praxim)	2.2 l/ha	220 l	III-IV	5.IV	1	6	1	1	25	150	150
9	Foliar feeding (Masterblad)	2.5 l/ha	250 l	V	10.V	1	6	1	1	25	150	150
10	Harvesting	2105 kg/ha	210.5 t	VII	6-9.VII	4	8	1	0	3.36	26.9	107.6

Table 2. Technological map for autumn sowing of coriander, after predecessor wheat

№	Technological operations	Agrotechnical requirements	Volume of work - V p , (ha, t, t.km, pcs.)	Agrotechnical period	Planned timeline from .... to	Number of working days - Dr	Duration of the working day - Td	Number of mechanics - nmex	Number of support workers - npr	Hourly productivity - Wh	Daily productivity - Wдн	Productivity for the planned period - W pl
1	Ploughing	18-20 cm	100 ha	VII-VIII	1-10.VIII	11	8	1	0	1.18	9.4	103.4
2	Discus	13-15 cm	100 ha	VIII-IX	1-3.IX	3	8	1	0	0.5	38.4	115.2
3	Cultivation	6-8 cm	100 ha	IX-X	25-27.IX	3	8	1	0	5.04	40.3	120.9
4	Sowing	15-20 kg/ha	2 t	IX-X	1-3.X	3	8	1	0	4.69	37.5	112.5
5	Fortified		100 ha	IX-X	1-3.X	3	8	1	0	6.75	54	162
6	Fertilization	150 kg/ha (N)	15 t	II-III	1.III	1	8	1	1	24.38	195	195
7	Spraying with herbicide (Praxim)	2.2 l/ha	220 l	III-IV	5.IV	1	6	1	1	25	150	150
8	Foliar feeding (Masterblad)	2.5 l/ha	250 l	V	10.V	1	6	1	1	25	150	150
9	Harvesting	2284 kg/ha	228.4 t	VII	6-9.VII	4	8	1	0	3.36	26,9	107.6

In 2020, 2021, 2022 on the Company's farm "Agro Impulse" Ltd. Voyvodinovo coriander yield - 2250-2130 kg/ha for autumn and spring sowing, respectively:
2500-2230 kg/ha
2100-1956 kg/ha

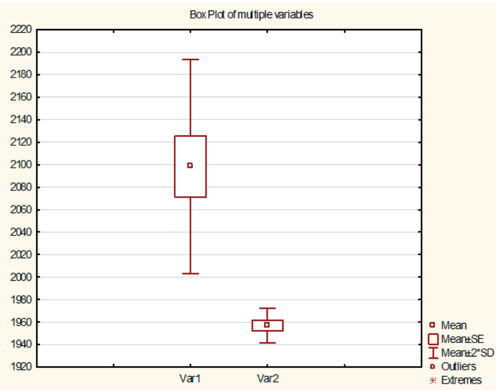

Figure 1. Multitiple variance

Mathematical analyses of both technologies will be performed with specialized data processing software.
When applying one-factor variance analysis, the influence of the factor (sowing term) on the parameter (yield) is established. The main idea in the application of this method is to see the reasons that cause a change in the parameter and in the decomposition of the general change in yield into two constituents - one caused by the factor of sowing time and the other by random and unreported factors.
With these two constituents, two independent estimates of the variance σ2 [ε] of the total disturbance effect are calculated ε (Mitkov et al., 1993)
By comparing these two estimates using Fisher's test, it is established whether the factor (sowing term) has a significant influence on the yield parameter or not.
Let the factor A be the time of sowing, and its levels are respectively A1 - autumn sowing and A2 - spring sowing, and let for both levels of the factor, three-year trials were conducted, which cause a change in the parameter Y - yield.
The data is entered in the following tabular form Table 3.

Table 3. Coriander yield at different sowing times

N of study	Factor levels	
	A1	A2
1	2250	2130
2	2500	2230
3	2100	1956
yi=∑yij	6850	6316
Ȳi	2283.3	2105.3

yi - Sums of all study;
Ȳi - average value.

Analysis of variance will be conducted at the significance level α = 0.05 (Table 4) (Figure 2)

Table 4. Univariate Test of Significance

Effect	Univariate Tests of Significance for Var2 (Spreadsheet1) Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	55646440	1	55646440	2672,558	0,000000
Var1	121807	1	121807	5,850	0,036142
Error	208214	10	20821		

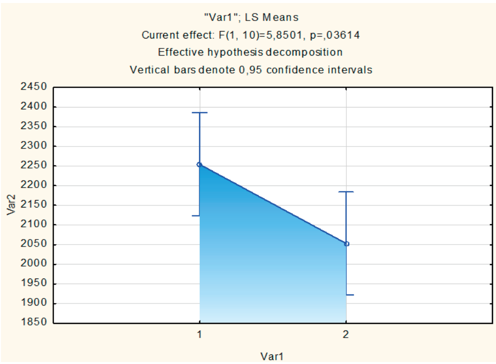

Figure 2. Efective hypothesis decomposition

The analysis shows that the sums of squares considered above and their respective degrees of freedom and variances are (Table 4):
SSA = 121807;
SSE = 208214;
SS = 121807+208214 = 330021.
Fisher's criterion F = 5.850 and its corresponding probability p = 0.0361 << 0.05 indicate that the factor A (sowing term) has a significant effect on the yield of coriander Y.
A T-test (Student's test) was also conducted to test the hypothesis of the samples (Table 5).
From the conducted inspection, it can be seen that there is a proven difference in yields depending on the applied cultivation technology.

Table 5. T-test for Independent Samples

Group 1 vs. Group 2	T-test for Independent Samples (2020) Note: Variables were treated as independent samples										
	Mean Group 1	Mean Group 2	t-value	df	p	Valid N Group 1	Valid N Group 2	Std.Dev. Group 1	Std.Dev. Group 2	F-ratio Variances	p Variances
Var1 vs. Var2	2245,000	2126,667	3,197679	4	0,032974	3	3	32,78719	55,07571	2,821705	0,523327

Group 1 vs. Group 2	T-test for Independent Samples (2021) Note: Variables were treated as independent samples										
	Mean Group 1	Mean Group 2	t-value	df	p	Valid N Group 1	Valid N Group 2	Std.Dev. Group 1	Std.Dev. Group 2	F-ratio Variances	p Variances
Var1 vs. Var2	2496,667	2220,000	5,187500	4	0,006572	3	3	65,06407	65,57439	1,015748	0,992187

Group 1 vs. Group 2	T-test for Independent Samples (2022) Note: Variables were treated as independent samples										
	Mean Group 1	Mean Group 2	t-value	df	p	Valid N Group 1	Valid N Group 2	Std.Dev. Group 1	Std.Dev. Group 2	F-ratio Variances	p Variances
Var1 vs. Var2	2098,333	1957,000	5,087430	4	0,007044	3	3	47,52192	7,549834	39,61988	0,049237

CONCLUSIONS

From the mathematical processing of the results obtained from the field tests, we can draw the following conclusions:

Through the use of analysis of variance and t-test to test the hypotheses of two independent samples at the level of significance, it was found that there is a proven difference in yields when growing coriander by the two technologies and the yield directly depends on the term of sowing.

The technology of autumn sowing of coriander gives proven higher yields compared to that of spring sowing.

Although spring sowing has higher production costs related to the preparation of the field for sowing and statistically proven lower yields of coriander, this sowing is recommended in a dry autumn.

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CHOROLOGY, ECOLOGY AND PHYTOSOCIOLOGY OF THE *Ruscus aculeatus* L. IN FOREST HABITATS FROM THE SOUTH OF OLTENIA, ROMANIA

Mariana NICULESCU

University of Craiova, Faculty of Agronomy, 19 Libertatii Street, Craiova, Romania

Corresponding author email: mniculescum@yahoo.com

Abstract

Ruscus aculeatus L. (Ruscaceae), is a shrub with strong, erect stems bearing numerous phylloclades widespread in western, southern and southeastern Europe, in Anatolia and northern Africa. This species is cited from few places in Oltenia. Following field research in the forest habitats of southern Oltenia, important populations of this species were identified. Such populations were identified in the lower Jiului basin, in the forest base of the Segarcea, Perişor and Craiova Forestry Districts. The most important populations with a large number of individuals, increased vitality and good conservation status are found in the Dâlga and Ţuglui arboretums. The species is found especially in the forests of the Quercus cerris and Q. frainetto, in the natural habitat - 91M0 Pannonian-Balkan oak - Oak forests (CLAS. PAL.: 41.76). Thirty populations of Ruscus aculeatus were identified and monitored in the Dâlga and Ţuglui arbotretums.

Key words: *Ruscus aculeatus, populations, corology, ecology, plant communities, forest habitats.*

INTRODUCTION

Ruscus aculeatus popularly called "thorn" is a plant known from Antiquity. It is part of the Liliaceae family and is a thorny subshrub, with rigid phyllocladia 1-4 cm long, with a thorn at the top. The plant is richly branched. The flowers of the thorn are yellow-white in color and are found in the shape of a star. The fruits are small red berries. It is found in forests or rare forests being a mesotrophic to eutrophic, xeromesophilic and thermophilic species. In Romania, the species is protected and considered a monument of nature (Ciocârlan, 2009).

In Europe, *Ruscus aculeatus* is most widespread around the mediterranean, native from North Africa (Morocco, Algeria, Tunisia and Libya) to Eastern Europe and Central Hungary (Tutin et al., 1964-1980, 1993).

In Romania, the species was cited from several places, for example: Vamanu Mountains, Bilbor village (Topliţa region); Şimleu hill, Băile 1 Mai, Hidişelul de Sus (Oradea region); on the hills in between the Şuncuiuş large valley and the Urman valley (Beiuş region); in Moneasa, along the Megieş valley, and also in Piatra Mică, Sebiş on the Pilişca hill, Dezna on the Corbului hill, Musteşti under the Drocea hill (Gurahonţ region); Cerna valley, Băile Herculane, Mehadia on the Străjuţ mountain, Danube valley in the Cazane place (Orşova region); Hinova on the Stârmina hill, Valea Hoţului (Turnu Severin region); Bucovăţ forest, Leamna de Jos, Palilula, Podari (Craiova region); Gâncioava (Segarcea region); Bechet (Corabia region); Rast (Băileşti area); Ponoare, Cloşani (Baia de Aramă zone); Vlădaia - inside the Bungetului forest (Vânju Mare region); Comana, located in Padina lui Vasile, and at the Fântâna cu Nuc, Călugăreni (in the Crucea de Piatră forest and also the Mihai Bravu forest) (Giurgiu region); Niculiţel - inside the forest (Tulcea region); Mangalia, above the thermal spa; Grozeşti, Slănic (Tg. Ocna region) (Maftei & Maftei, 2016).

In Oltenia, the species was also cited from Valea Motrului, Culmea Motrului, Dealurile Cerăngarilor, Trunchiul Cârceni (Costache, 2005).

This species is found in calcareous forests, thickets, shady rocky places and also wetlands. It needs mild temperatures in winter. *Ruscus aculeatus* can be found in a wide range of habitats listed in the Habitats Directive (Commission of the European Communities, 2009). This species is classified as having stable populations, but because in some areas of Romania the conditions of protection and maintenance of favorable conservation conditions for existing populations are not respected, it is likely that existing and future threats will cause the decline of several species in the near future.

MATERIALS AND METHODS

Study area

The field research regarding the species *Ruscus aculeatus* was carried out in the forest habitats around the towns: Dâlga, Leamna de Sus, Obedin, Mihăita, Tîmburești, Bratovoiești, Țuglui, Bucovăț, Bîzdâna, Almaj, Gogoșu, Ștefãnel (Dolj County), Rogova, Ogradena, Dubova, Svinita, Eibenthal (Mehedinți County), Negomir, Urdari, Artanu, Capul Dealului (Gorj).

Methods

To identify the species we looked into: *Romanian Flora*, vols. I-XII (1952-1976) and *Flora Europaea*, vols. I-V (1964-1980), *Illustrierte Flora von Mitteleuropa* (Hegi, 1987), *Atlas of North vascular plants: North of the Tropic of Cancer* (Hultén & Fries, 1986), *Red list of extinct, endangered, vulnerable vascular plants and rare from Romania's flora* (Boșcaiu et al., 1994).

For the analysis of the plant community in the study area was used the methoology of phyto-sociologic research of the Central European Phyto-Sociologic School, which is based on the principles and methods elaborated by Braun-Blanquet (1939). The plant communities were identified according to the characteristic, edifyng, dominant and differential species.

For the classification and phytosociology study were used synthesis papers on the Romanian (Coldea, 1991; 1997; Sanda et al., 1997) and Europeen vegetation (Géhu & Rivas-Martinez, 1981; Mucuna, 1997; Mucina et al., 2016; Rodwell et al., 2002; Raus et al., 2016). The name of the vegetal association was given taking into account the regulations stated by the Phytosociologic Nomenclature Code (Weber et al., 2000). The environmental analysis included altitude, slope, aspect, and soil properties.

RESULTS AND DISCUSSIONS

Ruscus aculeatus L. is known as a medicinal plant since antiquity, from a morphological point of view it is a sempervirescent shrub, with a horizontal rhizome on which metamorphosed stems with an assimilative role, called phyllocladia, are found. The leaves are reduced to membranous bracts. The white-green flowers are solitary and are inserted on the lower part of the phyllocladia. The fruits are red berries with 1-2 spherical seeds (Flora României, Vol. XI, 1976).

In our country, this species has been studied a lot from a phytopharmaceutical point of view, being an important plant for treating diseases of the circulatory system. *Ruscus aculeatus* has a protection regime in our country, the species being included on the red list of endangered vascular plants, in danger of extinction and, as a result. The branches of the plant are often collected by peasants and sold by the piece for decorative purposes. The plant is also used for medicinal purposes, thus not respecting the status of a protected plant.

Most populations were found in the Dâlga Forest and Lemna de Sus. In the Dâlga forest, 47 populations were inventoried and monitored. The number of individuals within these populations varies from 2 to 30 individuals. The state of conservation of the populations is very good, the individuals are well developed, they fruit very well and form large vigorous bushes (Figure 1).

Figure 1. *Ruscus aculeatus* in Dâlga Forest
(Photo: Mariana Niculescu)

Ecological and cenological characterization of the species Ruscus aculeatus **L.** *in the investigated territory*

Research carried out by us in habitats foresters highlighted the presence of some well-structured forest phytocoenoses floristically and cenotic, which belong to the plant communities: *Quercetum cerris* (Georgescu, 1941); *Quercetum frainetto-cerris* (Georgescu, 1945; (Rudski, 1949); *Carpino-Quercetum cerris* (Klika, 1934); *Quercetum frainetto* (Păun, 1966). Within the phytocoenoses analyzed, the species *Ruscus aculeatus* L. achieves a coverage between 5 and 60%. The greatest coverage is found in the phytocenoses on the sloping lands, with inclination between 15 and 25% and eastern exposure.

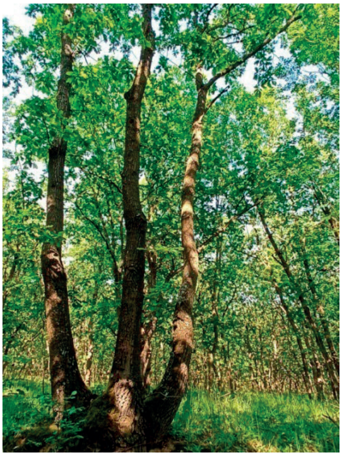

Figure 2. Aspect with the habitat of the *Ruscus aculeatus* species - Dâlga Forest

The populations of the Dâlga Forest belong to the plant community - *Carpino-Quercetum cerris* (Klika, 1934) (Table 1). The compaction of the canopy of the analyzed phytocoenoses in this forest is between 0.6-0.8. *Ligustrum vulgare, Euonymus europaeus, E. verucosa, Sambucus nigra, Rosa canina, Crataegus monogyna, Viburnum lantana* and *Cornus sanguinea* are frequently found in the shrub layer (Niculescu et al., 2009). The following species participate with high constancy in the composition of the floristic composition of the grass cover: *Festuca heterophylla, Potentilla micrantha, Tanacetum corymbosum, Vincetoxicum hirundinaria, Melica uniflora, Silene nutans, Hieracium sabaudum, Lathyrus vernus, Helleborus odorus, Asperula taurina, Lithospermum purpureo-coeruleum, Teucrium chamaedrys*.

From a conservative point of view, it was observed that the highest abundance-dominance of the species is within the Natura 2000 habitat - 91M0 Pannonian-Balkanic turkey oak - sessile oak forests; CLASS. PAL.: 41.76 (Gafta & Mountford, 2008) (Figure 2). In the forest habitats of the Leamna de Sus Forest, 16 populations with equally vigorous individuals, with very good fruiting, were found.

Ecological, phytosociological and population studies were done during the entire growing season to cover all morphological and phenological aspects.

Ruscus aculeatus populations show large numbers of individuals. In the Leamna de Sus Forest, the species was identified in the same type of habitat (Figure 3).

Figure 3. *Ruscus aculeatus* - Leamna de Sus Forest

Within this forest body, the species was identified in the plant community *Quercetum frainetto-cerris* (Georgescu, 1945; Rudski, 1949). The compaction of the canopy of the analyzed phytocenoses in this forest is between 0.6-0.7. *Euonymus europaeus, E. verucosa, Sambucus nigra, Crataegus monogyna, Viburnum lantana* are frequently found in the shrub layer. The following species participate with high constancy in making up the floristic composition of the grass blanket: *Helleborus odorus, Anemone nemorosa, Asparagus offcinalis, Asperula taurina, Lithospermum purpureo-coeruleum, Viola odorata* etc.

The species was inventoried and monitored without conducting population studies in these locations as well: Obedin, Mihăița, Tîmburești, Bratovoiești, Țuglui, Bucovăț, Bîzdâna, Almaj, Gogoșu, Ștefănel (Dolj County), Rogova, Ogradena, Dubova, Svinita, Eibenthal (Mehedinți County), Negomir, Urdari, Artanu, Capul Dealului (Gorj).

From a phytosociological point of view, within these locations the species was also identified in other Natura 2000 habitats: 40A0* Subcontinental peri-Pannonic scrub; CLASS. PAL.: 31.8B12p, 31.8B13, 31.8B14, 31.8B3p and Habitat 91I0* Euro-Siberian steppic woods with *Quercus* spp.

Table 1. *Carpino-Quercetum cerris* plant community (Klika, 1934)

No. of relevée	1	2	3	4	5	6	7	8	9	10	K
Altitude m.o.s. (x 10 m)	142	140	140	140	138	140	140	142	142	142	
Exposure	E	SV	E	SE	S	S	E	SE	E	SE	
Inclination (in grades)	10	10	15	10	15	20	25	20	10	25	
Canopy (%)	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.6	0.7	0.7	
Coverage of herbacaeous layer (%)	60	70	70	70	70	60	60	65	65	60	
Area (m^2)	400	400	400	400	400	400	400	400	400	400	
Char. ass.											
Quercus cerris	3-4	4	4	4	4	4	4	4	4	4	V
Carpinus betulus	1	+	1	1	1	+	+	+	+	+	V
Quercetea pubescenti-petraeae **et** ***Qurcetalia petraeae-pubescentis***											
Quercus frainetto	1	1	1	1	+-1	1	+	+	1	1	V
Rosa canina	+	+	+	+	+	+	+	+	+	+	V
Tanacetum corymbosum	-	+	-	+	-	-	+	-	+	-	II
Astragalus glycyphyllos	+	+	+	+	+	+	+	+	+	+	V
Ruscus aculeatus	**2**	**2**	**3**	**3-4**	**3-4**	**2**	**2**	**2**	**2-3**	**3**	**V**
Fragaria viridis	+	+	-	-	-	+	-	-	+	+	III
Vincetoxicum hirundinaria	+	+	+	+	+	+	+	+	+	+	V
Dianthus armeria	+	+	+	-	+	+	+	-	+	+	IV
Hypericum perforatum	+	+	-	-	+	+	-	-	-	-	II
Coronilla varia	+	-	+	-	+	-	+	-	-	+	II
Arabis turrita	+	+	-	-	-	+	-	-	+	+	III
Viola hirta	+	-	-	-	-	-	-	-	-	+	I
Poa angustifolia	+	-	-	+	-	-	-	-	-	-	I
Lithospermum purpureo- coeruleum	1	1	+-1	+	1-2	2	+	2	+-1	1	V
Festuca heterophylla	+	+	+	+	+	+	+	+	+	+	V
Teucrium chamaedrys	+	+	+	-	+	+	+	-	+	+	IV
Carex montana	+	+	-	-	+	+	-	-	-	-	II
Potentilla micrantha	+	-	+	-	+	-	+	+	-	+	III
Oryzopsis virescens	+	+	-	-	-	+	-	-	+	-	II
Potentilla alba	+	+	-	-	+	+	-	-	-	-	II
Lychnis coronaria	+	-	-	+	-	-	-	-	-	-	I
Fraxinus ornus	+	-	+	+	+	-	-	-	-	-	I
Helleborus odorus	+	+-1	1	1	+-1	+	+	+-1	+	+	V
Scutellaria altissima	-	+	+	+	-	-	-	+	+	+	III
Tilia tomentosa	+	+	+	+	+	+	+	-	-	+	IV
Acer tataricum	+	-	+	-	+	-	+	+	-	+	III
Querco Fagetea											
Cornus sanguinea	+	+	+	+	+	+	+	+	+	+	V
Crataegus monogyna	+	+	+	+	+	+	+	+	+	+	V
Viburnum lantana	+	+	+	+	+	+	+	+	+	+	V
Prunus spinosa	+	+	+	+	+	+	+	+	+	+	V
Cerasus avium	+	-	-	-	+	+	+	-	-	-	II
Ligustrum vulgare	+	+	+	+	+	+	+	+	+	+	V
Euonymus europaeus	-	+	+	+	-	-	-	+	+	+	III
Poa nemoralis	+-1	1	1	1	1	+-1	1	1-2	1	+-1	V
Smyrnium perfoliatum	1	1	+	-	+	-	1	1	+	-	IV
Veronica officinalis	+	+	+	+	+	+	+	+	+	+	V
Anemone nemorosa	+	1	1	1	1	+-1	1	1	1	+-1	V
Viola odorata	+	+	+	+	+	+	+	+	+	+	V
Viola alba	-	+	+	+	-	-	-	+	+	+	III
Melica nutans	+	+	+	+	+	+	+	+	+	+	V
Melica uniflora	+	+	+	+	+	+	+	+	+	+	V
Euphorbia amygdaloides	+	+	+	+	+	+	+	+	+	+	V

Veronica chamaedrys	+	+	+	+	+	+	+	+	+	+	V
Brachypodium sylvaticum	+	1	1	1	+-1	1	+-1	1	-	+	V
Viola odotata	+	+	+	+	+	+	+	+	+	+	V
Ranunculus auricomus	+	+	+	-	-	-	+	+	-	+	III
Lathyrus vernus	+	+	+	+	+	+	+	+	+	+	V
Hedera helix	+-1	1	1	1	+	+-1	+-1	+	+	+	V
Geum urbanum	+	+	+	+	+	+	+	-	-	+	IV
Asperula taurina	+	+	+	+	+	+	+	+	+	+	V
Hieracium sabaudum	+	+	+	+	+	+	+	+	+	+	V
Variae Syntaxa											
Lathyrus venetus	+	+	+	+	+	+	+	+	+	+	V
Ornithogalum umbellatum	-	-	-	+	+	+	-	-	-	-	II
Ornithogalum flavescens	+	-	-	+	-	+	-	-	-	-	II
Muscari comosum	-	+-1	-	+-1	+	-	1	-	-	+	III
Inula hirta	+	+	+	+	+	+	+	+	+	+	V
Orchis purpurea	-	-	-	+	-	+	-	-	-	+	II
Mycelis muralis	-	+	+	+	-	+	-	+	+	+	IV
Rubus caesius	-	-	-	+	+	+	-	-	-	-	II
Larthyrius hallersteinii	+	-	+	+	-	+	-	-	-	-	II
Euphorbia cyparissias	+	-	-	-	+	+	+	-	-	-	II
Betonica officinalis	+	-	-	-	+	-	-	-	-	-	I
Galium aparine	-	-	+	+	+	+	-	-	-	+	III
Viscaria vulgaris	+	+	-	-	-	+	-	-	-	-	II
Scrophularia nodosa	-	-	-	+	-	+	-	-	-	+	II

Place and data of the relevés: 1-10, Forest Dâlga, 28.IV.2020, 15.VI.2021, 12.V. 2022

CONCLUSIONS

Ruscus aculeatus is a protected species found on the IUCN Red List status of threatened species. In Romania it also has protection status. The species is found in forest habitats in a few places in the country. In the southern part of Oltenia, the species was identified in several forest habitats.

Following field studies, it was found that it is in a good state of preservation. The species was analyzed from a chorological, ecological and phytosociological point of view from several areas in Gorj and Dolj counties. Population studies were carried out in two locations: Dâlga Forests and Leamna de Sus (Dolj County), in these areas numerous populations with vigorous individuals and very good fruiting were found. Several pressures and threats to the conservation status of the species have also been observed, so sustainable, long-term measures are needed for this species.

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Agrostis capillaris L. - A REVIEW OF THE DISTRIBUTION, CHARACTERISTICS, ECOLOGICAL AND AGRONOMIC ASPECTS, AND USAGE

Marilena ONETE[1], Luiza-Silvia CHIRIAC[1], Roxana-Georgiana NICOARĂ[1], Florian Paul BODESCU[2], Minodora MANU[1]

[1]Institute of Biology Bucharest, 296 Splaiul Independentei Street, Bucharest, Romania
[2]SC Multidimension SRL, 7 Ciprian Porumbescu Street, Saveni, Ialomita, Romania

Corresponding author email: marilena.onete@gmail.com

Abstract

Agrostis capillaris L. (syn. A. tenuis Sibth.) is a perennial herbaceous, hemicryptophyte, native to Romania, distributed frequently from oak to subalpine levels. The circumpolar world distribution shows that the native range is quite extensive and can become adapted to a wide range of other habitats. Still, the literature review showed that in many countries, this species is introduced in grasslands as fodder for animals, as a sports turf and lawn species, and might be invasive in other countries replacing the native species from pastures and other grassland types. A. capillaris might be used as a hyper-accumulator, improving grassland quality; it provides economic value, social benefit, and environmental services.

***Key words**: Agrostis capillaris, A. tenuis, distribution, characteristics, ecological and agronomic aspects, usage.*

INTRODUCTION

Agrostis capillaris L., first published in Sp. Pl.: 62 (1753) is accepted as a species with 165 synonyms, the most used synonym in the literature is *A. tenuis* Sibth. It belongs to Poaceae Family which comprises over 12,000 accepted species (The Plant List, 2013). This perennial and rhizomatous geophyte grows primarily in the temperate biome and according to R.B.G.K. (2022) has a native range in Europe to N. China and Afghanistan, doubtfully present in Morocco and Tunisia and introduced in the Americas, Greenland, Australasia (Batson, 1998), East Asia and the Indian state of Arunachal Pradesh. It has environmental uses, as fodder for animals, sports turf, and as a lawn species. Vasey (1883) published *A. tenuis* as a new species of grass to the San Bernardino Mountains (California), Rapson & Wilson studied the floral phenology (1992a) and responses to light, soil fertility, and water availability (1992b) of *A. capillaris* as an invader in New Zealand (Rumball & Robinson, 1982; Edgar & Forde, 1991). As *A. capillaris* was introduced in New Zealand from Great Britain and comparing the genetic variation of *A. capillaris* within and between populations grown in comparable conditions in New Zealand and Great Britain over a very wide environmental range, Wilson & Rapson (1995) concluded that some of the evidence for non-adaptation in *A. capillaris* in New Zealand is caused by a small gene pool, and insufficient time for sorting of genotypes into habitats. Cho et al. (2016) documented the introduction of *A. capillaris* in Korea in disturbed areas, cemeteries, roadsides, and rough grassland. According to European databases, *A. capillaris* is shown as being a clonal species (Klimešová & Klimeš, 2019), indigenous, hemicryptophyte, with vegetative propagation by runners, storage organs and shoot metamorphoses, reproducing by seeds and vegetatively, its ecological strategy being "csr" (competitors/stress-tolerators/ruderals) (Grime et al., 1988), moderately to well tolerant of mowing, moderately tolerant of grazing and trampling, and with intermediate to high forage value. The studied species is found in many plant associations/habitats (Klotz et al., 2002): Class *Violetea calaminariae* (04.4: Communities of heavy metal soils), Class *Koelerio-Corynephoretea* (05.2: Pioneer communities of hair-grasses), Class *Festuco-Brometea* (05.3: Fescue-brome communities of dry and semi-dry grasslands), Class *Molinio-Arrhenatheretea*

(05.4: Commercially used grasslands), Class *Nardetea strictae* (05.6: Acidic mat-grass communities), Class *Melampyro-Holcetea mollis* (07.3: Acidophilous forest grassland ecotones), Class *Epilobietea angustifolii* (07.4: Communities of clear-felled areas), Class *Calluno-Ulicetea* (08.4: Heather and gorse heathlands), Class *Franguletea* (09.2: Acidic deciduous bush communities), Class *Carpino-Fagetea* (10.2: Mesophilous, summer-green deciduous forests), Class *Quercetea robori-petraeae* (10.3: Birch-oak forests).

According to Mucina et al. (2016) who proposed a vegetation classification widely used in Europe (for applied vegetation science, conservation planning and land management), plant associations comprising *A. capillaris* as diagnostic species are distributed as follow:

a. Order *Agrostio capillaris-Jasionetalia montanae* Foucault 1999 and Foucault 2001 in pioneer herb-rich vegetation on shallow soils on rocky outcrops in the nemoral and boreal zones of Europe (Order SED-02 *Sedo-Scleranthetalia* Br.-Bl. 1955)

b. Alliance *Agrostio capillaris-Peucedanion oreoselini* Reichhoff et Warthemann 2003 (syntax.syn.) Meso-xerophytic forest-edge communities on acidic soils in semi-shady to sunny habitats of temperate and (sub)boreal Europe (Alliance GER-05A *Melampyrion pratensis* Passarge 1979)

c. Alliance *Festuco-Agrostion capillaris* Redžić 1990 (1) in oligotrophic pastures in the lowland to submontane belts of the Western Balkans (Alliance NAR-01H *Achilleo-Arnicion* Horvat et Pawłowski in Horvat 1960)

d. Alliance *Nardo-Agrostion capillaris* Nordhagen 1936 and Nordhagen 1937 and Nordhagen 1943 in moderately chionophilous siliceous mat-grass swards of Scandinavia and as relicts in the Hercynian mountains (Alliance TRI-01B *Nardo-Caricion rigidae* Nordhagen 1943)

Maczey (2016) made a comprehensive and very interesting datasheet on *A. capillaris* covering identification, distribution, dispersal, hosts/ species affected, diagnosis, biology & ecology, environmental requirements, natural enemies, impacts, uses and prevention/control.

MATERIALS AND METHODS

We performed an internet search on plant lists presented online, using *Agrostis capillaris* as the main searchable word, followed by *A. tenuis*.

On Web of Science and Google Academic we used primarily *A. capillaris* and *A. tenuis* together with the words: distribution, characteristics, ecological and agronomic aspects, and usage. Secondarily we used a combination of these words. For access to so-called "grey literature" from the national level, we performed searches on Research Gate and Google Academic using the same method as before.

For distribution of the species at the national level, we used Romanian published books, especially Identification Manuals (Field Guides) for floras (Ciocârlan, 2009; Sârbu et al., 2013), habitats (Doniță et al., 2005; Gafta & Mountford, 2008) and plant associations (Sanda et al., 2008).

RESULTS AND DISCUSSIONS

Characteristics

Agrostis capillaris L. is a perennial tufted species, hemicryptophyte (geophyte) (Figure 1), oligotrophic - mesotrophic species with a circumpolar distribution.

Figure 1: *Agrostis capillaris* habit (left) (original) and clonality (right) (after Klimešová & Klimeš, 2019)

In Romania it is frequent from the oak forest to subalpine levels, in grasslands, shrublands or cleared forests (Ciocârlan, 2000). Ecological preferences of the species are: increased light, growing with difficulty in shade (L_7), with no particular preferences for temperature (euritherm)(T_x) and soil humidity (eurihydric) (U_x), soils of moderate to low acidity (R_5), soils poor in mineral nitrogen (N_3) (Sârbu et al., 2013). *A. capillaris* competes with other species for nitrogen (N) or phosphorus (P). Venterink & Güsewell (2010) conducted experiments showing competition with *Alopecurus pratensis* highlighting that *A. capillaris* is an equal or stronger competitor under P limitation and *A. pratensis* is a stronger competitor under N limitation. The competitive response of *A. capillaris* at the high N: P supply ratio was associated with low root mortality and high root phosphatase activity, but other factors might also be important in competition for P (root longevity, mycorrhizal hyphal length) (Miranda-Apodaca et al., 2020).

Soil microbial diversity (saprophytic, mutualistic, parasitic) has a very important role in ecosystem functioning and interactions with plant roots (Borozan et al., 2015). The seeds of *A. capillaris* can present cultivable endophytic bacteria and, on individuals growing on a long-term Cd/Ni-contaminated plot (Cd/Ni seeds), there are plant- and contaminant-dependent effects on the population composition, significantly improving plant growth. This indicates that inoculation of *Agrostis* with its seed endophytes might be beneficial for its establishment during phytoextraction and phyto-stabilisation of Cd-contaminated soils. (Truyens et al., 2014)

Distribution

The distribution of *A. capillaris*, is highly influenced by altitude and, due to its large ecological variability, it is distributed in the Banat Mountains in grasslands from low hills (237-340 m altitude), to high hills (468-545m altitude), submontane (709-722 m) and mountain levels (sub-level of beech forests and mixed beech and softwood forests), up to 1200 m average altitude, and even to 1446 m (Semenic Mountains, Mount Piatra Goznei) (Săndoiu et al., 2014). In well-lit places, *A. capillaris* is part of the dominant herbaceous species in grasslands: from the Transylvanian Plateau in Brașov County in Mercheașa - Homorod, at 530 m altitude, in Oak (*Quercus robur*) forest area and Jimbor - Homorod at 615 m altitude, in European wild pear (*Pyrus pyraster*) forest areas and from Gurghiului Mountains (Ibănești, Mureș County) at 1150 m altitude in Common beech (*Fagus sylvatica*) forest (Marușca et al., 2020). *A. capillaris* is typical of grassland where it is co-dominant with *Festuca rupicola* (Țucra et al., 1987) which is meso-xerophilic, moderately acidophilic, oligo-mesotrophic, medium tolerant to grazing, trampling, and mowing. The semi-natural grasslands forming the phytocoenosis of the woodland floor has mediocre agronomical value that supports a grazers density of 0.41-0.60 LU/ha, but it has considerable biodiversity importance because it includes many species with indicator values for High Nature Value grassland (Păcurar et al., 2020).

Grasslands with *Nardus stricta* typified by Plant Association *Violo declinatae-Nardetum* Simon 1966 have the widest distribution in higher mountainous regions of Romania (1700-2100 m). They comprise *A. capillaris* in their floristic composition because this species is a remnant from the *Festuco rubrae-Agrostetum capillaris* association that was replaced by *Violo declinatae-Nardetum* due to compaction and acidification of the soil. Following application of lime to increase soil reaction and overgrazing, plant associations with *A. capillaris* as a dominant, characteristic or diagnostic species (i.e. *Hypochoeri radicatae - Agrostetum tenuis* and *Festuco rubrae-Agrostetum capillaris*) are transformed into the Association *Hieracio pilosellae-Nardetum strictae* Pop et al. 1988, which is quite species-poor with soil rich in acidic humus and located in the Vlădeasa Mountains and Măgoaja Hills (Cluj County) at 700-1300 m altitude (Sanda et al., 2008).

In the Maramureș County, Associations *Hypochoeri radicatae-Agrostietum capillaris* Pop et al., 1988 and *Festuco rubrae-Agrostietum capillaris* Horv. (1951) 1952 are located on plateaux or slopes varying between 5 and 50°, showing various exposures, soils rich in organic matter and high variation in altitudes and soil pH, 620-1225 m and pH =

4.37-5.31, and 580-1268 m and pH 4.09-7.29 (Bărbos, 2006).

In areas of clearcut forests, very productive (about 4 t/year/ha) secondary meadows are formed with *Festuca rubra* and numerous other species in the upper levels of the sward and with *A. capillaris* in the ground cover (Doniță et al., 2005). In the *Festuco rubrae-Agrostetum capillaris* Association, *A. capillaris* dominates pioneer and fertilized areas; in *Hypochoeri radicata-Agrostetum tenuis* dominates on soils rich in organic materials. Once the input of nutrients in the soil increases, the *Festuco rubrae-Agrostetum capillaris* with *A. capillaris* as a dominant and characteristic develops into the Association *Lolio-Cynosuretum* Br.-Bl. et de Leeuw 1936 em. R. Tüxen 1937 where *A. capillaris* is a companion species (Sanda el al., 2008; Iliev & Bozhanska, 2023).

Some Romanian habitats (Doniță et al., 2005; Gafta & Mountford, 2008) have *A. capillaris* as a companion species, whilst in other habitats it is dominant or even characteristic (generally dominant or co-dominant species, which provide the largest volume of biomass and define the phytocoenosis) and diagnostic species (characteristics for the plant association from habitat) (Table 1).

Table 1. Romanian habitats, plant association, distribution, and characteristics of areal in which *A. capillaris* vegetate and its status (after Doniță et al., 2005; Gafta & Mountford, 2008)

Natura 2000 habitat	Romanian Habitat	Plant association	Distribution	Areas (ha)	Altitude (m)	Relief	Status of *A. capillaris*
4030 European dry heaths							
	R3112 South-eastern Carpathian heath of bilberry (Vaccinium myrtillus) and heather (Calluna vulgaris)						
		Vaccinio – Callunetum vulgaris Bük. 1942. (Syn.: Nardo – Callunetum Csürös 1964, Agrosteto – Callunetum Resmeriță et Csürös 1966, Arnica montana – Calluna vulgaris ass. Ghișa et al. 1970).					
			Western Carpathians, rare in the eastern Carpathians, at the altitude of beech and spruce forests				
				< 10	600–1750	moderately inclined slopes, manes.	companion species
40A0* Subcontinental peri-Pannonic scrub							
	R3121 Ponto-Pannonic scrub of blackthorn (Prunus spinosa) and spindle (Euonymus europaeus)						
		Euonymo – Prunetum spinosae (Hueck 1931) Tx. 1952 em. Pass. et Hoffim. 1968 (Syn.: Pteridio – Crataegetum monogynae Raâiu et Gergely 1979)					
			In Subcarpathian and Moldova, Muntenia, Transylvanian plateaux, sometimes along rivers in hilly areas, secondary in the place of clearcut sessile oak or mixed hornbeam and sessile oak forests				
				< 100	300–800	moderately inclined slopes	companion species
6240 *Sub-Pannonic steppic grasslands							
	R3413 Pannonic-Balkan meadows of Festuca rupicola and Cleistogenes serotina						
		Cleistogeno – Festucetum rupicolae (Soó 1930) Zólyomi 1958 corr. Soó 1964					
			Banat, Danube Clisura, Ardeal plaine				
				About 40.000	100–350	very slightly inclined slopes, flat ground.	companion species
	R3711 Dacian meadows of Nardus stricta and Molinia caerulea						
		Nardo-Molinietum Gergely 1958					
			Maramureș, Transylvania				
				20–25	350–400	Depressions with slightly inclined slopes	Among dominant species
6410 Molinia meadows on calcareous, peaty or clayey silt-laden soils (Molinion caeruleae)							
	R3713 Anthropogenic meadows of Juncus tenuis and Trifolium repens						
		Juncetum tenuis (Diemont, Siss. et Westhoff 1940) Schwik. 1944					
			River terraces and floodplains in Transylvania, Muntenia, Moldova				
				25–30	100–500	Plane or slightly inclined areas	companion species
6510 Lowland hay meadows (Alopecurus pratensis, Sanguisorba officinalis)							
	R3716 Danubian-Pontic meadows of Poa pratensis, Festuca pratensis and Alopecurus pratensis						
		Poetum pratensis Rãv., Cãzac. et Turenschi 1956, Ranunculo repentis – Alopecuretum pratensis Ellmauer 1933, Agrostideto-Festucetum pratensis Soó 1949					
			Rivers' floodplains from Transylvania, Banat, Oltenia, Muntenia, Dobrogea, Moldova.				
				1–2 (300–400)	100–350 (400)	River-terraces in the plain, flat areas or with very slightly inclined slopes	companion species
6520 Mountain hay meadows							
	R3803 South-eastern Carpathian meadows of Agrostis capillaris and Festuca rubra						
		Festuco rubrae – Agrostetum capillaris Horvat 1951.					
			Subcarpathian hills, Mehedinți and Transylvanian plateaux, Dorna Depression, Obcinele Moldovei.				
				400–500	350–700	low inclined slopes, southern and eastern expositions	Characteristic and diagnostic species

No Natura 2000 habitats							
	R3114 Cut forest with raspberry (Rubus idaeus)						
		Fragario – Rubetum (Pfeiffer 1936) Siss. 1946 (Syn.: fit. Impatiens noli-tangere Dihoru 1975)					
			All Romanian Carpathians, at the altitude of beech forests				
				> 100	700–1400	moderately inclined slopes	companion species
	R3801 South-eastern Carpathian meadows of Trisetum flavescens and Alchemilla vulgaris						
		Cerastio holosteoidis – Trisetum flavescenti Sanda et Popescu 2001 (Poo – Trisetum flavescetis auct. rom. non Knapp 1951), Trisetetum flavescentis (Schröter) Brockmann 1907					
			Lower and middle hilly levels of South-East Carpathians				
				10.000–12.000	650–800	Very low inclined slopes, northern or north-eastern exposition	companion species
	R3804 Dacio-Getic meadows of Agrostis capillaris and Anthoxanthum odoratum						
		Anthoxantho – Agrostetum capillare Silinger 1933.					
			Getic and Moldavian Subcarpathians, Transylvanian Plateau				
				500–600	300–700	Moderate inclined slopes	Characteristic and diagnostic species
	R4165 Forest glades with Betula pendula						
		Agrostis tenuis-Betuletum verrucosae Resmeriță 1970					
			Southern Carpathians: depressions within the mountains (Mestecănișul de la Reci in Covasna County).				
				< 100	490–530	sand dunes alternating with flat ground	Characteristic and diagnostic species

Ecological and agronomic aspects

Permanent grasslands in Romania are sometimes subject to bad management and unfavourable climatic conditions leading to an important decrease in productivity. Grassland degradation consists of changes in plant environment and vegetation structure. The current strategy at national level is primarily to save resources and protect the environment, and secondarily to increase yields (Samuil et al, 2011). External factors affect plant species that respond to them in different ways depending on their biotic (other species coexisting in the same ecosystem) and abiotic interactions (Hart et al., 2009; Miranda-Apodaca et al., 2020).

When applying different types and levels of organic fertilisation and depending on climatic conditions, there is a positive effect of organic fertilisers on sward structure, biodiversity and productivity in permanent grasslands dominated by *A. capillaris*. Thus, the management of organic fertilisers did not affect the biodiversity of these grassland types (Samuil et al., 2008; Razec et al., 2009; Damgaard et al., 2011). The application of nitrogenous fertilisers stimulates growth and depresses P concentration in *A. capillaris* (Minson, 2012). At lower altitudes, mineral N accumulated in the soil almost entirely as nitrate and at highest altitude as ammonium (Harrison et al., 1994).

For semi-natural grassland vegetation characterised by *A. capillaris* and *Festuca rubra*, another category of management might be mowing at least once in every 4-5 years to preserve their floristic structure and avoid the agronomic and ecological degradation of their vegetation caused by abandonment (Păcurar et al., 2015). Grazing and mowing, as a mixed management practice, might still degrade the pasture because these grasslands are dominated by species sensitive to grazing and medium resistance to trampling (Voşgan et al, 2015).

Individuals of *A. capillaris* exposed to previous root herbivory had significantly more infection by arbuscular mycorrhizae than individuals with no exposure (Johnson et al., 2013, Borozan et al., 2015).

In Călimani National Park, on waste dump deposits resulting from mining activities, the natural reconstruction (without human intervention) of the plant communities started (primary vegetation type) with *A. capillaris* (among other species) as the dominant species with a clumped distribution (Oprea et al., 2008).

Plants from sites with a high heavy metal concentration adapt more easily to new contaminants. *A. capillaris* shows two types of tolerance: induced or constitutional. Genetically based induced tolerance occurs in individuals after long-term contact with high concentrations of metals in the soil, resulting in contamination-resistant populations due to selection pressure (Budak et al, 2006). Constitutional tolerance occurs in individuals not stressed by selection, and when in contact with high concentrations of heavy metals in the soil develop tolerances depending on species, variety, and developmental phase. Individuals living on soils rich in heavy metals have developed two methods of dealing with contamination (Fitter & Hay, 2012; Kraj et al., 2021):

1. avoidance of the stress factor throughout reducing the quantity of metal uptake from the soil by changing the pH in the rhizosphere, secreting organic acids that limit the availability of metals, mycorrhizae, etc.
2. accumulation throughout uptake of the contaminants and storing them in the roots and maintaining low content in the shoots.

Testing for copper tolerance on populations of *A. capillaris* collected from copper mines grasslands (Cu.Gr.) and uncontaminated nearby ordinary pasture (NonCu.Gr.) showed that: a) growing on Cu.Gr., individuals are copper tolerant; b) individuals from NonCu.Gr. growing very close to Cu.Gr. show absence of copper tolerance; c) the character of copper tolerance has high heritability (McNeilly & Bradshaw, 1968; Whiteley & Williams,1993; Bes et al., 2013).

Usage

Grasslands are important as global sinks and sources of atmospheric carbon (Murray et al., 2004). Grasslands comprising *A. capillaris* are exploited for mowing and grazing in a semi-extensive mode, their areas being under-exploited (Cirebea et al., 2016) or in an intensive mode, their areas being over-exploited (Nicoară et al., 2020; Onete et al., 2020; 2021).

These types of grasslands, traditionally managed, keep their high species diversity thus have high nature conservation value. Their conservation depends on many factors, including the response of the plant species composition to changes in climate variables. In these grasslands, *A. capillaris* and *F. rubra* exchange co-dominance in response to changes in temperature and humidity as the major factors in climatic fluctuations (Păcurar et al., 2014).

For usage of grasslands containing *A. capillaris* as pasture and haymaking, we must consider the method and period of harvest because these factors might affect the increase in yield and crude protein content (Iliev et al., 2022)

Heavy-metal mine areas are naturally colonised by metal-tolerant (Kiss et al., 1998), pseudo-metallophyte (Pandey et al., 2023), or facultative metallophyte (Minson, 2012) ecotypes of *A. capillaris* which can be used for phyto-extraction and phyto-stabilisation of polluted sites with the addition of bark compost as well as mycorrhizae (Truyens et al., 2014).

The best result was found for the combination of water works granules and mycorrhiza. At the end of the vegetative growth season, the solution concentration of most environmentally critical elements was below those of untreated systems (Ietswaart et al., 1992; Macklon et al., 1994; Malcová et al., 2003; Karlsson et al., 2012; Williams et al., 2013; Nicoară et al, 2014).

The efficiency of phytoremediation is achieved by altering some soil characteristics (pH, precipitation, binding compounds or stimulating redox levels). These soil characteristics can act both synchronously or independently. For instance, Pb is precipitated as phosphate in the roots of *A. capillaris* (Emam et al., 2021).

CONCLUSIONS

Agrostis capillaris L. (syn. *A. tenuis* Sibth.) is a perennial herbaceous, hemicryptophyte, clonal plant species. The circumpolar distribution shows that the native range is quite extensive and can become adapted to a wide variety of other habitats. *A. capillaris* is a native species in Europe (except the Balearic Islands, Corsica, Sardinia, Sicily and including European Russia west of the Urals) and Asia, has status doubtful in Morocco and Tunisia (Africa), is not native in most of Asia, Australasia, North and South America. The literature review showed that in many countries, this species is introduced in grasslands as fodder for animals, as sports turf and a lawn species, and might be invasive in other countries replacing the native species of pastures and other grassland types. The studied species is met in many plant associations and habitats according to both foreign and Romanian literature. In Romania, *A. capillaris* is native and forms semi-natural permanent grasslands or pioneer vegetation types on waste dumps and is naturally distributed from hill to mountainous and subalpine regions, in many plant associations as a companion species and in others as a characteristic and diagnostic species.

Grasslands comprising *A. capillaris* are used for grazing or mowing, and their maintenance in space and time is strongly correlated with impact intensity.

A. capillaris might be used as a hyper-accumulator, improving grassland quality, as well as providing economic value, social benefit, and environmental services.

The management of permanent grasslands, via usage, type of control and period and intensity of fertilisation, has a great influence on productivity, species composition and structure of vegetation, species dominance and biodiversity (species of plants, underground and above-ground invertebrates, and vertebrates). Natura 2000 habitats identified by plant associations comprising *A. capillaris* are multiple at national and international levels, showing the necessity of habitats conservation.

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THE REMANENT EFFECT OF THE APPLICATION OF SEWAGE SLUDGE COMPOST ON AGRICULTURAL LAND UPON THE SOIL PROPERTIES AND PEA CROP

Eusebiu SAFTA[1, 2], Leonard ILIE[2]

[1]Urban Services for Mioveni Community, 4 Carol Davila Street, Arges County, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: eustil@yahoo.com

Abstract

The compost obtained from the 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 quality of the soil through the supply of nutrients and organic matter necessary for the practice of modern, ecological agriculture, under the conditions of improving the capacity to retain moisture in the soil, also reducing the pressure on the environment generated by the storage of these wastes. The compost used in the experiments is suitable for use in agriculture without risks of environmental and soil pollution, in compliance with the norms in force. The obtained results highlight the fact that by applying compost, even in the variants where the highest doses were applied (60 t/ha), there are no significant changes in the chemical properties of the soil, especially in the content of heavy metals. The values determined in the soil after applying the compost to all the tested variants are far below the maximum values allowed for the concentrations of heavy metals in the soil. Also, analyzing the results regarding the risk of translocation of the different chemical elements in the pea grains, it can be seen that, in general, all the indicators register values far below the limits from which zootoxicity phenomena can occur. There was no increase in the content of heavy metals in the pea grains, as the doses of compost used increased.

Key words: *sewage sludge, compost, remanence effect, pea, soil properties.*

INTRODUCTION

The changes in the soil as a result of the application of these residues are recorded by the agrochemical state, the agrophysical state, the agrobiological state, all competing to define the fertility of the soil. The positive effect of organic materials in general, and of those originating from urban activity, in particular, on the physical, chemical and biological properties of the soil also affects plant production, which in most cases registers increases. The organic matter is directly involved in the retention of heavy metals, Cu being one of the first metals studied in this regard (Kiikkilia et al., 2002) showing that the biosolid is an immobilizing agent for this heavy metal. On the other hand (Moolenaar & Beltrami, 1998) demonstrated the fact that heavy metals can also be complexed by dissolved organic matter, which influences the ion balance. One of the main factors involved in the absorption of heavy metals is soil pH, their accessibility being very reduced in the reaction range of 6.5-7. The presence of competitive metal ions can affect the adsorption of heavy metals in soils. Ca^{2+} 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 through cationic exchange reactions, while Cu and Pb form organic complexes with Fe, Al and Mn oxides (Kiekens, 1983). Adsorption of heavy metals by iron oxides is accompanied by protonation being dependent on pH, according to the research carried out by Cornell & 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 structuring processes of the elementary soil particles in hydrostable aggregates, to the increase in the water retention capacity. The concentration of heavy metals is among the most important factors that restrict the use of urban waste products on agricultural land, due to their potentially negative effects on plant biomass and their translocation into food products.

Data from literature contain different ways of interpreting the contents of heavy metals in soils, specifying limit values, but it seems that the closest model to reality is the one that takes into account the content of total forms in the soil (EPA, 1993). The current acidity of the soil recorded a tendency to decrease through fertilization with biosolids in the years of application and retention.

The potential acidity followed the same variation as the current one, so that under the conditions of biosolid application and in the first year of retention, it had a tendency to decrease, so that later in the third year of retention there was a re-actualization (Trașcă et al., 2008).

The increased interest in soil fertilization with sludge resulting from urban wastewater has been manifested since 1970, when it was established that it can be considered an organic fertilizer (Tomlin et al., 1993).

The use in agriculture of the sludge resulting from the treatment of urban wastewater is dependent on the properties of the soil among which pH, the content of organic matter and nutrients 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 et al., 2000).

The effect of sludge from urban wastewater treatment on the soil is investigated both from the point of view of soil improvement and from the point of view of the impact on the environment. As Beltran et al. (1999) pointed out, knowledge of the chemical composition of sludge is of particular importance when making recommendations on application rates to agricultural land.

Over time, soluble organic compounds tend to pass into insoluble forms, the amount of heavy metals settling at low values when bioavailability decreases (McBride, 1995).

The researches on the effect of sludge application on the soil has not exceeded 30 years, as demonstrated by numerous works (Kabata-Pendias, 2004).

MATERIALS AND METHODS

In order to study the remanence effect in the 3rd year from the application of a compost resulting from the sewage sludge proceeding from the treatment plant on the agricultural crops and on the soil properties, pea was used as a test plant (the compost was applied in 2018).

The administration of the compost in the doses specific to each variant was carried out by manual spreading and incorporated into the soil by plowing. Basic soil work and normal plowing at a depth of 25 cm.

The experience included 5 experimental variants in 3 repetitions, the area of an experimental plot being of 105 m^2. The experimental variants were: V_1 - Control; V_2 - 10 t/ha; V_3 - 20 t/ha; V_4 - 40 t/ha; V_5 - 60 t/ha.

The researches were performed on a soil of luvosol, podzolite, 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 land (Traşcă et al., 2008).

The quality of the compost used in the experiments

The qualitative parameters of the analyzed compost are within acceptable values for its use in agriculture, including in terms of heavy metal content (Table 1) (Safta & Ilie, 2022).

The effect of applying compost from sewage sludge as a fertilizer in agriculture is currently focused on cultivated plants and soil.

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-Gogoașă 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).

RESULTS AND DISCUSSIONS

The effect of compost application (remanence effect in the 3rd year) on pea plants

It can be noted that, in general, for all the analyzed indicators, there are no values that are phytotoxic for the field pea plants as a result of the remanence effect, the 3rd year of fertilization with sewage sludge compost in increasing doses.

Table 1. The main chemical characteristics of compost

No.	Parameter	Value	Maximum values (Ord. 344/2004)
1	Volatile substances (%)	35.34	-
2	pH	7.09	-
3	$C_{organic}$ (% d.m.)	21.5	-
4	N_{total} (% d.m.)	1.52	-
5	P_2O_5 (% d.m.)	1.38	-
6	K_2O (% d.m.)	0.675	-
7	CaO (% d.m.)	0.35	-
8	Cadmium (mg/kg d.m.)	1.04	10
9	Chromium (mg/kg d.m.)	44.8	500
10	Copper (mg/kg d.m.)	74.3	500
11	Nickel (mg/kg d.m.)	26.5	100
12	Lead (mg/kg d.m.)	46.3	300
13	Zinc (mg/kg d.m.)	612	2000
14	Cobalt (mg/kg d.m.)	6.34	50
15	Arsenic (mg/kg d.m.)	4.09	10
16	Total coliform bacteria (probable no./g d.m.)	1352400	-
17	Fecal coliforms (probable no./g d.m.)	236523	-
18	Enterococci (UFC/g d.m.)	105840	-

Only for copper and lead, slight increases are observed, at high doses of compost, compared to the unfertilized variant, but without affecting the normal growth, development and fruiting of field pea plants, these being well below the concentration limits at which phenomena of toxicity occur (Table 2).

We will proceed to follow how the translocation of different chemical elements took place in the field pea grains, by analyzing their content, after harvesting and interpreting these values in correlation with the contents determined in the leaves.

Table 2. Influence of the remanence effect of compost application on the chemical composition of pea leaves - at flowering

No.	Parameter	V_1	V_2	V_3	V_4	V_5
1	Humidity (%)	67.8	84.6	85.0	75.9	87.43
2	Cadmium (mg/kg d.m.)	<0.3	<0.3	<0.3	<0.3	<0.3
3	Chromium (mg/kg d.m.)	<1.5	<1.5	<1.5	<1.5	<1.5
4	Copper (mg/kg d.m.)	7.67	7.49	7.14	6.81	7.98
5	Nickel (mg/kg d.m.)	4.47	2.76	4.64	2.65	2.31
6	Lead (mg/kg d.m.)	2.97	5.03	3.41	5.62	7.14
7	Zinc (mg/kg d.m.)	32.79	28.21	31.58	26.11	30.27
8	Cobalt (mg/kg d.m.)	<1.5	<1.5	<1.5	<1.5	<1.5
9	Arsenic (mg/kg d.m.)	<0.03	<0.03	<0.03	<0.03	<0.03

It can be noted that, in general, for all the analyzed indicators, no toxic values are recorded for the field pea grains as a result of the remanence effect, the 3rd year of fertilization with sewage sludge compost in increasing doses (Table 3).

The concentrations in the pea grains, resulting from the variants that received increasing doses of compost from sewage sludge, are normal and similar to those in the control variant.

The influence of compost fertilization (remanence effect in the 3rd year) on the soil

The very high values for the physical indicator, bulk density, for all variants, including the control without compost (2.39 g/cm^3), mean an exaggerated settlement of the soil, by performing mechanical works at high humidity (Table 4). It is necessary to follow this indicator, when analyzing the soil samples taken after harvesting the field pea crop.

Table 3. Influence of the remanence effect of compost application
on the chemical composition of pea grains - at harvest

No.	Parameter	V_1	V_2	V_3	V_4	V_5
1	Humidity (%)	9.35	10.49	11.16	10.45	11.33
2	Cadmium (mg/kg d.m.)	<0.3	<0.3	<0.3	<0.3	<0.3
3	Chromium (mg/kg d.m.)	<1.5	<1.5	<1.5	<1.5	<1.5
4	Copper (mg/kg d.m.)	4.26	5.04	4.38	5.14	4.86
5	Nickel (mg/kg d.m.)	<1.5	<1.5	<1.5	<1.5	<1.5
6	Lead (mg/kg d.m.)	<1.5	<1.5	<1.5	<1.5	<1.5
7	Zinc (mg/kg d.m.)	41.65	25.68	31.58	33.25	47.07
8	Cobalt (mg/kg d.m.)	<1.5	<1.5	<1.5	<1.5	<1.5
9	Arsenic (mg/kg d.m.)	<0.03	<03	<0.03	<0.03	<0.03

It can be seen from the data presented in Table 4, that in the 3rd year after the application of increasing doses of compost from sewage sludge, concentrations of heavy metals above the maximum allowed values are not found in the soil, not even with high doses of compost (Table 5). Practically, there is already a uniformity of these concentrations, in all variants, close to the level of the non-fertilized variant, which shows that these otherwise very low concentrations are not related to the application of increasing doses of compost from sewage sludge.

Table 4. The influence of the remanence effect of compost application on the chemical characteristics
of the soil before pea sowing

No.	Parameter	V_1	V_2	V_3	V_4	V_5
1	pH	6.31	6.23	6.21	6.24	6.34
2	Organic matter content (%)	3.91	3.65	3.70	4.10	3.70
3	Soluble salts (%)	0.012	0.013	0.011	0.011	0.013
4	Water storage capacity (%)	34.8	34.8	34.9	36.2	33.5
5	Bulk density (g/cm^3)	2.39	2.19	2.36	2.23	2.23
6	Total C (% d.m.)	1.06	1.05	1.02	1.02	1.10
7	N_{total} (% d.m.)	0.113	0.115	0.114	0.118	0.115
8	P_2O_5 (% d.m.)	0.114	0.129	0.112	0.115	0.135
9	K_2O (% d.m.)	0.90	0.86	0.90	0.60	0.90
10	CaO (% d.m.)	0.25	0.28	0.27	0.25	0.27
11	Cadmium (mg/kg d.m.)	<0.3	0.4	<0.3	0.32	<0.3
12	Chromium (mg/kg d.m.)	41.63	41.29	42.36	35.62	42.34
13	Copper (mg/kg d.m.)	18.12	17.07	16.99	16.17	16.99
14	Nickel (mg/kg d.m.)	25.77	24.28	24.78	23.39	24.41
15	Lead (mg/kg d.m.)	17.71	15.90	15.15	13.84	15.36
16	Zinc (mg/kg d.m.)	61.82	66.11	62.27	60.75	66.54
17	Cobalt (mg/kg d.m.)	12.95	13.06	12.50	11.66	13.98
18	Arsenic (mg/kg d.m.)	0.055	0.052	0.041	0.051	0.057
19	Total coliform bacteria (probable no./g d.m.)	21 386	659340	113988	13563	66474
20	Fecal coliform (probable no./g d.m.)	4403	142450	98	863	0
21	Enterococci (UFC/g d.m.)	13	0	0	12	0

Table 5. The maximum permissible values for the concentrations of heavy metals in soils

The analyzed parameter	The limit value (mg/kg d.m.)
Cadmium	3
Copper	100
Nickel	50
Lead	50
Zinc	300
Mercury	1
Chromium	100

(Source: Order 344/2004)

It is noted that the very high values of the physical indicator, bulk density, are maintained in all variants, including the control variant without compost (2.53 g/cm^3), it means an exaggerated settlement of the soil, by performing mechanical works at high humidity (Table 6).
It is also found from the data presented, that in the third year after the application of increasing doses of compost from sewage sludge, concentrations of heavy metals above the maximum allowed values are not found in the soil, not even with high doses of compost (Table 5).
Practically, there is already a uniformity of these concentrations, in all variants, close to the level of the non-fertilized variant, which shows that these otherwise very low concentrations are not related to the application of increasing doses of compost from sewage sludge.

Table 6. The influence of the remanence effect of compost application on the chemical characteristics of the soil after pea harvesting

No.	Parameter	V_1	V_2	V_3	V_4	V_5
1	pH	6.38	6.38	6.24	6.17	6.32
2	Organic matter content (%)	3.54	3.56	3.38	4.95	3.61
3	Soluble salts (%)	0.014	0.011	0.009	0.013	0.014
4	Water storage capacity (%)	34.7	35.4	33.3	34.7	34.7
5	Bulk density (g/cm^3)	2.53	2.28	2.31	2.40	2.18
6	Total C (% d.m.)	1.30	0.89	1.16	1.14	1.16
7	N_{total} (% d.m.)	0.109	0.104	0.108	0.120	0.121
8	P_2O_5 (% d.m.)	0.119	0.124	0.118	0.121	0.148
9	K_2O (% d.m.)	1.22	1.07	1.18	0.85	1.22
10	CaO (% d.m.)	0.23	0.31	0.29	0.27	0.32
11	Cadmium (mg/kg d.m.)	<0.3	0.35	<0.3	<0.3	<0.3
12	Chromium (mg/kg d.m.)	58.71	55.23	55.18	51.47	52.76
13	Copper (mg/kg d.m.)	19.08	18.16	20.04	17.96	18.05
14	Nickel (mg/kg d.m.)	26.25	27.19	25.37	23.85	22.57
15	Lead (mg/kg d.m.)	20.13	16.17	14.03	14.03	17.11
16	Zinc (mg/kg d.m.)	60.41	63.34	64.73	64.13	63.58
17	Cobalt (mg/kg d.m.)	13.51	14.62	10.90	12.07	11.46
18	Arsenic (mg/kg d.m.)	0.049	0.050	0.038	0.047	0.052
19	Total coliform bacteria (probable no./g d.m.)	512	241	5772	5746	5707
20	Fecal coliform (probable no./g d.m.)	0	0	0	0	0
21	Enterococci (UFC/g d.m.)	0	0	0	1	0

CONCLUSIONS

The studied compost lends itself to use in agriculture without the risk of environmental and soil pollution with the rigorous observance of the entire set of specific technical measures.
For the field pea crop (3rd year), it can be noted that in general, for all the analyzed indicators, no toxic values are recorded for the field pea grains as a result of the remanence effect, 3rd year of fertilization with sewage sludge compost in increasing doses.
The concentrations in the pea grains, resulting from the variants that received increasing doses of compost from sewage sludge, are normal and similar to those in the control variant.
It is also found from the data presented, that in the third year after the application of increasing doses of compost from sewage sludge, concentrations of heavy metals above the maximum allowed values are not found in the soil, not even with high doses of compost.
Practically, there is already a uniformity of these concentrations, in all variants, close to the level of the non-fertilized variant, which shows that these otherwise very low concentrations are not related to the application of increasing doses of compost from sewage sludge.
It should be mentioned that in order to comply with the strict rules known in agricultural research, for the accuracy of the obtained experimental results, it is recommended to carry out another three-year rotation cycle, with

a second application of the compost, only in this way can the combined effect of the directly applied compost be verified, with its remanence effect, especially on the soil, but also indirectly on the plants.

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CYTOGENETIC STUDIES IN *Amaranthus retroflexus* - CHROMOSOME NUMBERS AND PHYLOGENETIC ASPECTS

Aida Patricia SĂRĂCIN[1], Larisa Marina Elisabeth CHIRIGIU[2], Ion SĂRĂCIN[3], Johny NEAMȚU[1], Ioan Alexandru SĂRĂCIN[3]

[1]University of Medicine and Pharmacy, Faculty of Pharmacy, 200349, Craiova, Romania
[2]"Constantin Brancusi" University, Faculty of Medical and Behavioral Sciences, 210135, Targu Jiu, Romania
[3]University of Craiova, Faculty of Agronomy, 19 Libertatii Street, Craiova, Dolj County, Romania

Corresponding author email: saracin.alex@gmail.com

Abstract

The study of the cell and cell division is of great importance, because it makes it possible to identify the genetic material, the mechanism by which genes are transmitted from the mother cell to the daughter cells, from ascendants to descendants, how genetic recombination is carried out and how they are produced mutations at gene level, as well as restructuring at chromosome level. The study of the preparations is carried out in bright light under a microscope. For the study of the chromosomes of the Amaranthus retroflexus species (2n = 34), the fast Feulgen-Rossenbeck staining method was used, which uses a bleached basic fuchsin solution (Schiff's reagent) as a dye.

Key words: *cell, genetic, chromosomes, Amaranthus retroflexus.*

INTRODUCTION

The cell (lat. *celulla* = room) constitutes the fundamental morpho-physiological unit of living matter and has a very complex structure.

The study of the cell and cell division is of great importance, because it makes it possible to identify the genetic material, the mechanism by which genes are transmitted from the mother cell to the daughter cells, from ascendants to descendants, how genetic recombination is carried out and how they are produced mutations at gene level, as well as restructuring at chromosome level (Chadoeuf-Hannel, 1982; Schonbeck, 1980).

The variety of cells existing in the living world is extremely large, however, it was possible to establish a general scheme of the cell structure, valid for all organisms, consisting of membrane, cytoplasm and nucleus (Ahrens & Stoller, 1983; Allemann et al., 1996).

The cell membrane - represents the external covering of the cell, through which the separation and communication with the external environment is achieved (Drzewiecki, 2001, Horak et al., 2000). It has, in most plants, a skeletal and selective role, regulating the circulation of substances towards and outside the cell (Brenner et al., 2000; Horváth, 1991; Gutterman et al., 1992).

The cell membrane also has a physiological and biochemical role, through its participation in the regulation of cellular metabolism, ensuring substance exchanges from one cell to another through a system of canaliculi (plasmodesmata) (Buhler et al., 1996, Bürki et al., 2001, Ferreira et al., 1991).

The permeability of the membrane, with the help of which the exchange of substances is carried out, represents one of the most complex functions of the membrane (Eberlein et al., 1992; McLachlan et al., 1995; Pandey, 1999).

Cytoplasm represents the cellular headquarters contained between the cell membrane and the nuclear one. It consists of a relatively homogeneous semi-viscous liquid, which forms a colloidal system (Buhler et al., 1996; Bürki et al., 2001; Ferreira et al., 1991). From a chemical point of view, proteins, lipids and carbohydrates are found in the cytoplasm, and besides these, mineral salts and water are also present (Aguyo, 2000; Greizerstein, 1992; Senesac, 1985).

The nucleus - is usually in the middle of the cell and is surrounded by the nuclear envelope. It is a spherical or ovoid corpuscle present in almost all plant and animal cells and measures 1/4-1/3

of the volume of a cell (Aellen, 1959; El Aydam, 1997; McWilliams et al., 1968). Inside is the karyolymph, the nuclear chromatin made up of nucleoproteins (DNA and histone and non-histone proteins), one or more nucleoli, ribosomes (Chadoeuf-Hannel, 1981).
The electronic microscope is an optical magnifying device that uses the photon as a source of radiation, an element of the spectrum of electromagnetic waves (Chadoeuf-Hannel, 1983; Schonbeck, 1981).
Cytological examination in photon microscopy is limited by the power of separation or resolution of the apparatus, the most valuable quality of a microscope. It is believed that the maximum limit for the resolving power of the photon microscope is 0.2 microns, a value that cannot be exceeded due to the long wavelength of the photon.
By fitting special devices to the ordinary electron microscope, it is possible to achieve:
- phase contrast microscopy;
- microscopy in fluorescent light;
- microscopy on a dark background;
- microscopy in polarized light.
The usual electron microscope consists of three parts: mechanics, optics and the light source.

Figure 1. The Kruss type electron microscope, used in the laboratory for viewing microscopic preparations of the *Amaranthus retroflexus* species (2n = 34)

The main reason for the study is to understand the structure and functions of cells which leads to the progress in technology, such as biotechnology and genetic engineering, which have many practical applications in medicine, industry and agriculture**.**

MATERIALS AND METHODS

The study of the preparations is carried out in bright light under a microscope (Figure 1). Place the preparation on the microscope table, adjust the sharpness for the 10x objective and look for the desired structure. A dividing cell is brought to the center of the microscopic field. Raise the condenser a little and switch to higher objectives (20x, 40x, 60x, etc.). Usually, the study of mitotic and meiotic cell division is performed with the 40x and 60x objective.
In order to study the entire preparation, one starts from one part of it and walks in front of the objective from one end to the other of the slide and from bottom to top until all the cellular structures are covered.
For the study of the chromosomes of the *Amaranthus retroflexus* species (2n=34), the fast Feulgen-Rossenbeck staining method was used, which uses a bleached basic fuchsin solution (Schiff's reagent) as a dye.
In this sense, the following stages were completed:
Obtaining the biological material. Chromosomes are visible only during the phases of cell divisions. Thus, they can be highlighted in the young meristematic tissues at the tips of roots, stems or young leaves. They can also be highlighted during meiosis, during the formation of gametes.
In order to obtain meristematic tissues from the species *Amaranthus retroflexus*, seeds of the respective species were germinated in disposable plastic pots, on moist filter paper, as well as on a support of 100% cotton disks, moist (Figures 2 and 3) (of mentioned that, in the case of the latter variant, the germination percentage was much better).

Figure 2. *Amaranthus retroflexus* seeds germinated on filter paper

Figure 3. *Amaranthus retroflexus* seeds germinated on 100% moist cotton pads

In order to keep a humid atmosphere inside the pot and to prevent the penetration of sunlight, filter paper and wet cotton pads were also placed on the cover of the germination pots, in order to promote germination. When the roots reached a length of about 8-10 mm, they were harvested with tweezers, moving to the next stage.

Fixation. It has the role of killing the cells and ensuring the coagulation of the cellular constituents, avoiding as much as possible the modification of the internal and external structure of the cells. Acetic acid 45% was used as a fixative (glacial acetic acid 45% and distilled water 55% at a temperature of 20C. The ampoules with the biological material were then kept in the refrigerator for 24 hours (Figure 4).

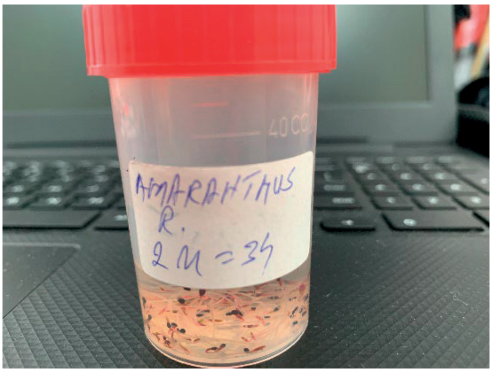

Figure 4. The biological material consisting of meristematic roots of *Amaranthus retroflexus*, in the fixation stage

Hydrolysis. It has the role of macerating the tissues by partially dissolving the intercellular pectic substances, thus facilitating the coloring process. It was performed in a solution of normal HCl at a temperature of 60°C, for 15 minutes.

Coloring. It was performed with a bleached basic fuchsin solution (Schiff's reagent), with selective staining at the chromosomal chromatin level.

The temporary preparations were analyzed under the optical microscope during the next 2-3 hours, until complete staining of the cytoplasm (Figure5).

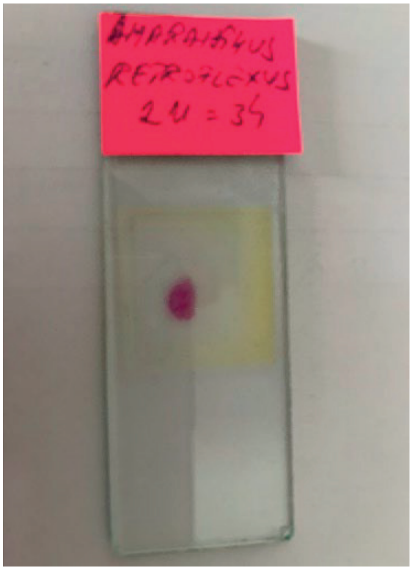

Figure 5. Temporary microscopic preparation of *Amaranthus retroflexus* prepared for viewing

a

b

c

Figure 6. The normal appearance of mitosis in the species *Amaranthus retroflexus* (60x), highlighted in some stages: a- interphase; b-prophase; c-telophase

RESULTS AND DISCUSSIONS

The preparation of meristematic roots from *Amaranthus retroflexus* using the described method yielded suitable material for the study of mitotic cell division. The Feulgen-Rossenbeck staining method successfully highlighted the chromosomes in the cells, enabling their observation under the microscope. The use of a 40x or 60x objective was found to be suitable for studying mitotic and meiotic cell division in this species (Figure 6).

The obtained results demonstrate the effectiveness of the described method for preparing and staining meristematic tissues from *Amaranthus retroflexus*. The use of acetic acid as a fixative and HCl for hydrolysis proved to be appropriate for preserving the cellular structure and facilitating the staining process. The selective staining of chromosomal chromatin with Schiff's reagent allowed for clear observation of the chromosomes under the microscope.

The successful observation of mitotic and meiotic cell division in *Amaranthus retroflexus* provides valuable information for understanding the cellular mechanisms involved in the growth and reproduction of this plant species. Further studies using this method could explore variations in the chromosome number or structural abnormalities in different individuals or populations of *Amaranthus retroflexus*, contributing to a better understanding of the genetics of this species.

CONCLUSIONS

The study of cells and cell division is indeed crucial for understanding many biological processes. Through this study, we can identify genetic material and mechanisms of inheritance, such as mitosis and meiosis, and how they contribute to genetic recombination and mutations.

The *Amaranthus retroflexus* species, with a chromosome number of 2n = 34, can be studied using various methods, including staining techniques. The Feulgen-Rossenbeck staining method is a widely used technique that involves staining DNA with Schiff's reagent, a bleached basic fuchsin solution. This staining technique allows for the visualization of the chromosomal material within the cell, providing insights into the structure and behavior of chromosomes during cell division.

Overall, the study of cells and cell division, as well as the use of staining techniques like the Feulgen-Rossenbeck method, can help researchers better understand the genetic processes that underlie many biological phenomena.

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THE INFLUENCE OF PLANT HORMONAL COMBINATION OVER DIFFERENT PARAMETERS OF GROWING AND DEVELOPMENT FOR POTATO PLANTLETS

Andreea TICAN, Mihaela CIOLOCA, Monica POPA

Institute of Research and Development for Potato and Sugar Beet Brasov,
2 Fundaturii Street, Brasov, Romania

Corresponding author email: tican_andreea@yahoo.com

Abstract

The main objective of this experience was monitoring the effect of plant growth regulators and their combinations on potato plantlets development, respectively on the following parameters: plantlets height, leaves number, root length, weight of fresh plantlet and of root. Thus, during in vitro multiplication process, two auxins were tested: naphthalene acetic acid (NAA) and indolyl acetic acid (IAA) (in two concentrations 0.05% and 0.1%, for both auxins), together with a gibberellin: gibberellic acid (GA3) (0.02%) and in vitro behavior was observed for three potato varieties: Marvis, Castrum, Ervant under the influence of these growth hormones. The nutrient medium supplemented with IAA 0.05% determined plantlets obtaining with high height and with the highest value of fresh root weight. The culture medium containing 0.1% NAA was effective in forming the number of leaves, showing a positive influence. NAA 0.1% + 0.02% GA3 and IAA 0.05% + 0.02% GA3 combinations had a beneficial effect on root length and fresh plantlets weight.

Key words: *hormonal combination, in vitro, multiplication, potato, plantlets.*

INTRODUCTION

Micropropagation can be defined as in vitro clonal multiplication through tissue culture and rapid multiplication of plant material in order to obtain a large number of descending plants (Mohapatra, 2017).
Rapid multiplication is an extensive method used to increase the nuclear stock of seed potatoes. This technique is widely used in many countries (Murashige, 1974; Hussey, 1981) is very flexible and offers a high rate of multiplication. Also, by rapid multiplication, it is possible to produce the disease-free seed potato from the disease-carrying seed (Roca et al., 1978; Wang, 1982).
In general, the effectiveness of micropropagation depends on the explants and their source, the treatment of the explants during their preparation for *in vitro* culture, the composition of the culture media, the micropropagation and the performance of the regenerated seedlings. The organ that it will the source of tissue depends on the physiological or ontogenic age of the organ, the season in which the explants are taken, their size and the general quality of the parent plant (Murashige, 1974, cited by Mohapatra & Batra, 2017).
For plantlets production on the nutrient medium, different techniques have been used over the years, the basic methods being similar in most laboratories and is based on the rapid growth of a minicutting on a sterilized liquid or solid culture medium (Ranalli, 1997).
Minicuttings with a single leaf are inoculated on the surface of a solid medium (Espinoza et al., 1984). The axillary/apical buds grow quickly, so in 3-4 weeks a new plantlet regenerates that can be further subculture in a fresh environment. The use of nodal cuttings in vitro is probably the most common method of propagation, applied in the early stages of commercial seed potato production (Pruski, 2007).
Very important factors in micropropagation are: the photoperiod, the temperature in the growth room, the balanced combination of growth hormones. Successful *in vitro* multiplication depends on the presence of an appropriate combination of auxins with gibberellic acid (GA3) in the propagation medium (Kumlay, 2014). Roest and Bokelmann, cited by Kumlay, 2014, suggested that a lower concentration of auxin with GA3 (0.25 mg l^{-1}) had a positive impact on the development of potato plantlets shoots and roots. Zhang et al. (2005) (cited by Kumlay, 2014) suggest that increasing plantlets

length has been promoted among potato explants with increasing concentrations of IAA; however, the stimulatory effect of IAA was enhanced by the addition of GA3.
Auxins are involved in many physiological processes: they interact with other endogenous substances and, of course, with other phytohormones, especially cytokinins, gibberellins and ethylene (Cachiță-Cosma & Sand, 2000). Auxin synthesis is located in the very young leaves, in the active buds. At low concentrations, physiologically stimulating, auxins exert a beneficial effect on growth, while at higher concentrations, they can become toxic (Cachiță-Cosma, 2000).
The physiological action of gibberellins, in general, can be summarized as follows: gibberellins elongate the internodes of the stems; plays an important role in regulating the endogenous level of auxin, etc. (Cachiță-Cosma, 2000).

MATERIALS AND METHODS

This study took place in the Tissue Culture Laboratory of National Institute of Research and Development for Potato and Sugar Beet Brasov, Romania. During in vitro multiplication process, an experiment was performed with reference to the influence of two auxins: naphthalene acetic acid (NAA) and indolyl acetic acid (IAA) (in two concentrations 0.05% and 0.1%, for both auxins), together with a gibberellin: gibberellic acid (GA3) (0.02%).
Thus, the bifactorial experience (3 x 8), on 3 repetitions had the following factors: experimental factor A - variety, with three graduations: a1 - Marvis, a2 - Castrum, a3 - Ervant and experimental factor B - multiplication medium (with 8 graduations): - b1 - naphthalene acetic acid (0.05%); b2 - naphthalene acetic acid (0.1%); b3 - indolyl acetic acid (0.05%); b4 - indolyl acetic acid (0.1%); b5 - naphthalene acetic acid (0.05%) with gibberellic acid (0.02%); b6 - acetic naphthalene acid (0.1%) with gibberellic acid (0.02%); b7 - indolyl acetic acid (0.05%) with gibberellic acid (0.02%); b8 - indolyl acetic acid (0.1%) with gibberellic acid (0.02%).
The experimental variants can be seen in the Figure 1.

Organizing the experience:

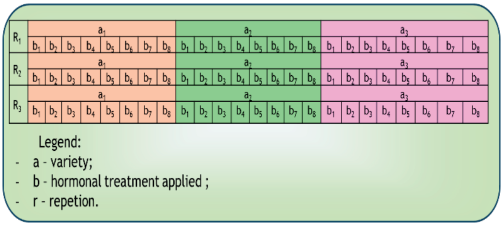

Figure 1. The scheme of experimental variants arranging

Microplants are used in seed potato production system. From healthy developed microplants (Figure 2) from each internode were obtained minicuttings (Figure 3).

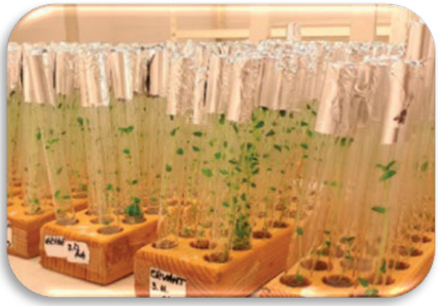

Figure 2. Developed plantlets

Figure 3. Minicuttings

All operations must be performed in the laminar air flow hood (Figure 4). The axillary/ apical buds inoculated (Figure 5) grow rapidly, so that in 3-4 weeks a plantlet regenerates which can be further subcultured on a fresh medium. After 30 days from the minicuttings inoculation on the 8 variants of culture medium, the following determinations were performed: plantlets length (cm), number of leaves/plantlets, root length (cm), fresh plantlet weight (mg) and weight fresh root (mg).

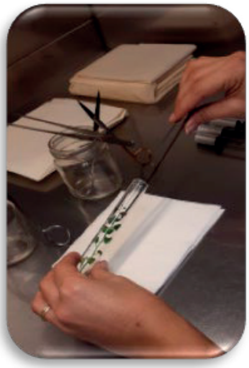

Figure 4. Multiplication in the laminar air flow hood

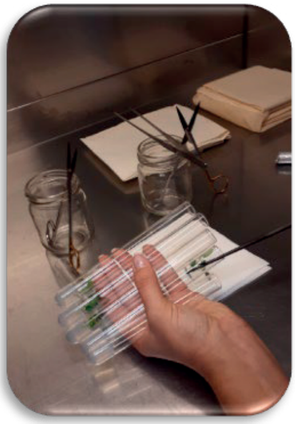

Figure 5. Inoculation of minicuttings

RESULTS AND DISCUSSIONS

Results were analyzed using MSTAT-C statistical package. Differences among the means were compared by the Duncan's Multiple Range Test at 1 level of significant.

Table 1 shows the behavior of the three experimented varieties, analyzed through the prism of the Duncan test. Regarding the height of the plantlets, Castrum variety (10.02 cm) can be distinguished, which differs significantly from the Marvis and Ervant varieties (7.92 and 7.31 cm).

For the second element analyzed, respectively, the number of leaves, Marvis variety (10.54) stands out, a variety that differs significantly from the Castrum variety (8.08 leaves).

Castrum and Marvis varieties (5.13 cm and 4.92 cm) stood out for their root length, differing significantly from the Ervant variety (2 cm).

Examining the results in terms of plantlets weight, we draw attention to the Marvis variety, which had a high capacity in plantlets mass formation (253.81 mg), differing significantly from the varieties Castrum and Ervant (170.96 and 120.68 mg).

From the analysis of the variety's behavior regarding the fresh root weight, Marvis and Castrum varieties can be noticed, which determine obtaining of high values (95.73 mg and 90.42 mg), detaching significantly from the Ervant variety (50.79 mg). Marvis and Castrum varieties have also been identified with high values in terms of root length.

Nutrient variants containing (Table 2) indolyl acetic acid (0.05%) together with gibberellic acid (0.02%), naphthalene acetic acid (0.05%), indolyl acetic acid (0.05%) had a positive influence on plantlets formation, which determined plantlets development with high height (9.83 cm; 9.44 cm; 9.22 cm), favorable in the process of multiplication. Naphthalene acetic acid (0.05%) together with gibberellic acid (0.02%) showed a strong inhibitory effect on plantlet growth (6.78 cm).

The hormonal combinations studied did not have a significant effect on plantlets formation leaves. By using 1% naphthalene acetic acid, plantlet with the highest number of leaves were obtained (10.44), so this hormone had the greatest beneficial influence in leaf formation, although there were no significant differences between nutritive medium variants. The lowest value is observed when applying NAA (0.1%) + GA3 (0.02%) combination in the culture medium, but as it is mentioned above there are no statistical differences between the variants with hormonal treatments.

The hormonal combination of naphthalene acetic acid 0.1% and gibberellic acid (0.02%) induced the formation of a root with the highest value of root length (5.06 cm), without differing from the combinations: naphthalene acetic acid (0.05%) - gibberellic acid (0.02%): 4.94 cm; naphthalene acetic acid (0.1%): 4.78 cm and naphthalene acetic acid (0.05%): 4.56 cm. The lowest value was recorded when applying indolyl acetic acid (0.1%) to the culture medium: 2.61 cm without statistically differentiating it from the influence of indolyl acetic acid (0.1%) and gibberellic acid (0.02%): 3.11 cm.

The combination of indolyl acetic acid (0.05%) and gibberellic acid (0.02%) hormones had as effect plantlets obtaining with the highest weight (237.22 mg), followed by the combination of indolyl acetic acid (0.1%) and gibberellic acid (0.02%) (214.57). At the opposite pole was using of 0.1% naphthalene acetic acid in the nutrient medium, leading to the lowest value of plantlets weight.

The highest value of root weight was recorded when supplementing the culture medium with indolyl acetic acid (0.05%): 106.68 mg. The minimum value of the root weight was obtained under the influence of 0.05% naphthalene acetic acid: 44.86 mg.

The maximum value of plantlets height was determined for the Castrum variety (13.17 cm), by using in the culture medium the hormonal combination IAA (0.05%) + GA3 (0.02%) (Table 3), without statistically differentiating by value recorded by applying NAA 0.05% (12.67 cm).

NAA (0.05%) + GA3 (0.02%) combination had a negative influence on plantlets formation for the Ervant variety (5.67 cm), strongly affecting their growth.

Regarding leaves number/plantlets, 0.1% naphthalene acetic acid was effective, resulting in the highest value (13) for Marvis variety. For the Ervant variety, a positive influence (11 leaves) showed indolyl acetic acid (0.1%), without significantly differentiating by the effect of naphthalene acetic acid on leaf formation for Marvis variety. IAA 0.05% plant growth regulator and the combination NAA (0.05%) + GA3 (0.02%) had a beneficial effect in leaf formation for Castrum variety, without statistical difference. Instead, using NAA (0.1%) + GA3 and IAA (0.1%) + GA3 combinations had a very pronounced negative effect on leaf formation for Castrum and Ervant varieties (7 leaves).

Regarding the root length, the highest value is observed for Castrum variety (7.5 cm) by applying NAA (0.1%) + GA3 (0.02 %) combination. Also, this hormonal combination, had a positive influence on Marvis variety, without statistically differentiating the recorded values (6.3 cm). For Ervant variety, NAA (0.1%) + GA3 (0.02%) treatment had a negative effect, inhibitory on plantlets root formation (1.3 cm).

The combined influence of variety and treatments with growth regulators performed on plantlets weight highlights the IAA (0.05%) + GA3 (0.02%) treatment, for Marvis variety, leading to the highest value (352.1 mg). The Castrum variety is distinguished by a high plantlets weight (257.3 mg) by applying the same treatment as for Marvis variety, respectively IAA (0.05%) + GA3 (0.02%). For this parameter, 0.1% NAA had a negative influence on the Castrum variety (92.1 mg).

Combined influence examination for variety and hormonal treatments on root weight reveals the beneficial effect of the combination IAA (0.05%) + GA3 (0.02%) for the Marvis variety, resulting in the highest value (140.1 mg). For Castrum and Ervant varieties, IAA growth regulator 0.05% showed a positive influence (128.9 mg and 102.9 mg), without statistical difference. At the opposite pole is the effect of NAA (0.1%) + GA3 combination for Ervant variety which recorded the lowest value of root weight (10.2 mg).

Table 1. The influence of genotype on the elements of growth and development under the influence of *in vitro* treatments with growth regulators, NIRDPSB Brasov (2022)

Variety	Plantlet's height (cm)	Leaves number	Root length (cm)	Weight of fresh plantlet (mg)	Weight of fresh root (mg)
Marvis (a_1)	7.92 B	10.54 A	4.92 A	253.81 A	95.73 A
Castrum (a_2)	10.02 A	8.08 B	5.13 A	170.96 B	90.42 A
Ervant (a_3)	7.31 B	9.46 AB	2.00 B	120.68 B	50.79 B
	LSD 5%=1.29	LSD 5%=1.56	LSD 5%=0.63	LSD 5%=63.87	LSD 5% = 31.33

For each column, the averages followed by the same letter are not significantly different according to the Duncan multiple comparison test ($p < 0.01$).

Table 2. The influence of growth hormones in the culture medium on the elements of growth and development, NIRDPSB Braşov (2022)

Plant regulators growth treatment	Plantlet height (cm)	Leaves number	Root length (cm)	Weight of fresh plantlet (mg)	Weight of fresh root (mg)
NAA (0.05%) (b_1)	9.44 A	9.22 A	4.56 A	179.74 BCD	44.86 D
NAA (0.1%) (b_2)	7.78 CD	10.44 A	4.78 A	141.1 D	66.19 BCD
IAA (0.05%) (b_3)	9.22 A	10.22 A	3.72 B	165.59 CD	106.68 A
IAA (0.1%) (b_4)	8.89 AB	9.33 A	2.61 C	194.29 BC	93.17 ABC
NAA (0.05%) +GA_3 (0.02%) (b_5)	6.78 D	8.89 A	4.94 A	153.77 CD	72.58 ABCD
NAA (0.1%) + GA_3 (0.02%)(b_6)	7.94 BC	8.67 A	5.06 A	168.17 CD	60.94 CD
IAA (0.05%) +GA_3 (0.02%) (b_7)	9.83 A	9.33 A	3.33 B	237.22 A	87.33 ABC
IAA (0.1%) + GA_3 (0.02%) (b_8)	7.44 CD	8.78 A	3.11 C	214.57 AB	100.09 AB
	LSD=1.09	LSD=2.09	LSD=0.81	LSD=41.97	LSD=34.22

For each column, the averages followed by the same letter are not significantly different according to the Duncan multiple comparison test ($p < 0.01$).

Table 3. The combined influence of variety and growth hormones used in the culture medium on the elements of growth and development, NIRDPSB Braşov (2022)

Variety	Plant regulators growth treatment	Plantlet's height (cm)		Leaves number		Root lenght (cm)		Weight of fresh plantlet (mg)		Weight of fresh root (mg)	
Marvis	NAA (0.05%)	8.3	DEF	11	ABC	5.7	BC	242.3	BCD	58.9	DEFG
	NAA (0.1%	7.8	DEF	13	A	6.3	AB	190.0	CDEFG	90.7	ABCDE
	IAA (0.05%)	8.7	DE	12	AB	4.0	DEF	233.9	BCDE	88.3	ABCDE
	IAA (0.1%)	8.7	DE	9	BCD	2.7	FGHI	236.1	BCDE	67.7	CDEFG
	NAA (0.05%) + GA_3	7.0	EFG	9	BCD	5.8	BC	219.8	BCDEF	94.7	ABCDE
	NAA (0.1%) + GA_3	7.5	DEFG	9	BCD	6.3	AB	264.2	B	93.9	ABCDE
	IAA (0.05%) + GA_3	8.7	DE	12	AB	4.5	CDE	352.1	A	140.1	A
	IAA (0.1%) + GA_3	6.7	FG	11	ABC	4.0	DEF	292.1	AB	131.5	AB
Castrum	NAA (0.05%)	12.67	A	8	CD	6.0	B	179.5	DEFGH	55.7	DEFG
	NAA (0.1%)	7.67	DEF	8	BCD	5.8	BC	92.1	J	49.9	DEFG
	IAA (0.05%)	12.00	AB	9	BCD	5.2	BCD	160.2	FGHIJ	128.9	AB
	IAA (0.1%)	10.67	BC	8	CD	3.2	EFGH	178.9	DEFGH	128.2	AB
	NAA (0.05%) + GA_3	7.67	DEF	9	BCD	6.3	AB	138.3	GHIJ	73.1	BCDEF
	NAA (0.1%) + GA_3	7.17	EFG	7	D	7.5	A	123.7	GHIJ	78.7	BCDEF
	IAA (0.05%) + GA_3	13.17	A	8	CD	3.7	EF	257.3	BC	82.2	ABCDE
	IAA (0.1%) + GA_3	9.17	CD	8	CD	3.3	EFG	237.6	BCDE	126.5	ABC
Ervant	NAA (0.05%)	7.33	DEFG	9	BCD	2.0	GHI	117.4	GHIJ	19.9	FG
	NAA (0.1%)	7.83	DEF	10	ABCD	2.2	GHI	141.4	GHIJ	57.9	DEFG
	IAA (0.05%)	7.00	EFG	10	ABCD	2.0	GHI	102.7	IJ	102.9	ABCD
	IAA (0.1%)	7.33	DEFG	11	ABC	2.0	GHI	167.9	EFGHI	83.5	ABCDE
	NAA (0.05%) + GA_3	5.67	G	9	ABCD	2.7	FGHI	103.2	IJ	49.9	DEFG
	NAA (0.1%) + GA_3	9.17	CD	10	ABCD	1.3	I	116.6	HIJ	10.2	G
	IAA (0.05%) + GA_3	7.67	DEF	9	BCD	1.8	HI	102.3	IJ	39.7	EFG
	IAA (0.1%) + GA_3	6.50	FG	7	D	2.0	GHI	114.0	HIJ	42.3	EFG
		LSD=1.9		LSD=3.6		LSD=1.4		LSD=72.7		LSD=59.3	

For each column, the averages followed by the same letter are not significantly different according to the Duncan multiple comparison test ($p < 0.01$).

CONCLUSIONS

The effect of hormonal combinations in the multiplication process varied from cultivar to cultivar, according to the parameters studied.

The combination of IAA (0.05%) + GA3 (0.02%) resulted in a pronounced growth of plantlets for the Castrum variety. The NAA 0.1% growth regulator favoured the Marvis variety in leaf formation. Examination of the results regarding the root length suggests the high capacity of Castrum variety to form them, determining the highest value by using NAA 0.1% + GA3 0.02%.

For IAA (0.05%) + GA3 (0.02%) combination there is a positive influence on the weight of plantlets and their root for the Marvis variety.

The nutrient medium supplemented with IAA 0.05% determined plantlets obtaining with high height and with the highest value of fresh root weight. The culture medium containing 0.1% NAA was effective in forming the number of leaves, showing a positive influence.

NAA 0.1% + 0.02% GA3 and IAA 0.05% + 0.02% GA3 combinations had a beneficial effect on root length and fresh plantlets weight.

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THE BIOMASS QUALITY OF COMMON NETTLE, *Urtica dioica* L., AND ITS POTENTIAL APPLICATION IN MOLDOVA

Victor ȚÎȚEI

"Alexandru Ciubotaru" National Botanical Garden (Institute), 18 Padurii Street, MD 2002, Chisinau, Republic of Moldova

Corresponding authors email: vic.titei@gmail.com

Abstract

Common nettle, Urtica dioica L., is an herbaceous perennial forb belonging to the Urticaceae family, distributed in temperate and tropical region in many parts of the world, and it has been reported to have multiple uses. We investigated the quality of the biomass of the local ecotype of Urtica dioica grown in the experimental plot of the "Alexandru Ciubotaru" National Botanical Garden (Institute), Chisinau, Republic of Moldova. The results revealed that the dry matter of the common nettle whole plant harvested in flowering period contained 20.7% CP, 10.6% ash, 26.8% CF, 32.9% ADF, 57.4% NDF, 6.6 % ADL, 26.3% Cel, 24.5% HC, with forage value 633 g/kg DDM, RFV= 103, 12.47 MJ/kg DE, 10.23 MJ/kg ME and 6.25 MJ/kg NEl. The ensiled mass contained 22.1% CP, 14.5% ash, 28.3% CF, 33.8% ADF, 53.0% NDF, 6.6 % ADL, 27.3% Cel, 19.2% HC, with forage value 626 g/kg DDM, RFV= 110, 12.34 MJ/kg DE, 10.13 MJ/kg ME and 6.14 MJ/kg NEl. It has been determined that studied common nettle substrates have C/N=13.4-15.0 and the biochemical methane potential reaches 319-321 l/kg ODM. The local ecotype of Urtica dioica can be used as an alternative forage source for farm animals or as co-substrate in biogas generators for renewable energy production.

Key words: *biomass, biomethane, green mass, silage, Urtica dioica.*

INTRODUCTION

The genus *Urtica* L. belongs to the family *Urticaceae* Juss. which comprises 46 accepted species names, 3 species occur in Bessarabia. The most prominent members of the genus are stinging nettle *Urtica dioica* L., which is distributed in temperate region in many parts of the world: Europe, Africa, Asia and North America. It isan herbaceous perennial plant, growing about 2 m in height, with square, green and erect stem, covered with stinging hairs with hooked protrusions. The soft green leaves are 3 to 15 cm long, opposite. The leaves have a strongly serrated margin, with rounded or cordate base and acute or acuminate leaf apex. The inflorescences are axillary, spiked, by four per node, with many small, green, unisexual flowers. The flowering period lasts from June to October; it is a wind pollinated plant. The fruits are oval, about 1.5 mm long and 1 mm wide, bilaterally compressed, with thin, grey-green or brown pericarp, with remaining Perigonia lacinia. The root system reaches depths of 30 cm in the soil, it consist of yellowish and cylindrical rhizomes and stolons. Stinging nettle can reproduce vegetatively and by seeds. It produces abundant seeds, 5000-20000 seeds per shoot, the seeds can germinate since the first days after reaching maturity. The stinging nettle is a nitrophilous plant that grows in a wide range of habitats, as a common species of riparian habitats, swamps, meadows, riverbanks, wastelands, floodplains, and disturbed areas, is present around the margins of arable fields and gardens (Kavalali, 2004; Mirza, 2010; Bisht et al., 2012; Di Virgilio et al., 2014; Gînju, 2020).

Nettle has been used as a folk medicine and as a food and forage sources from a long time. All morphological parts of nettle - stem, leaves, roots and seeds are utilized to produce many added-value natural products. It has been used frequently for its medicinal properties since the Bronze Age (3000-2000 B.C.) as a traditional herbal remedy in treatment of variety of diseases as gout, nephritis, haematuria, jaundice, menorrhagia, anemia, eczema, arthritis. Other research into nettle indicates that it has very high antioxidant potential, but is also known for a wide range of other activities such as antimicrobial, antiulcerogenic, analgesic, diuretic, antidiabetic, anti-inflammatory and anti-rheumatic (Teleuță et al., 2008; Biesiada et al., 2010; Bisht et al., 2012; Di Virgilio et al., 2014; Janet al., 2017; De Vico et al., 2018; Kregiel et al., 2018; Devkota et al., 2022; Tarasevičienė et al., 2023).

Stinging nettle has gained both commercial and scientific interest due to its multipurpose character. *Urtica dioica* is a valuable herbaceous plant and can be used for feed purposes as a feed additive for livestock, especially poultry (Egorov, 2014; Adhikari et al., 2015; Alieva et al., 2016; Milosevic et al., 2021; Kosolapov et al., 2022). According to Totev (1964) the chemical composition of *Urtica* hays was 2l-23% CP, 3-5% EE, 35-39% NFE, 9-2l% CF, l9-29% ash.

Producing industrial biomass on marginal lands, which are unsuitable for food production, might help mitigate potential conflicts between food and non-food production. Stinging nettle has a further card to play in this context, as it grows vigorously everywhere, without intensive inputs such as pesticides, herbicides, or irrigation, even in fairly poor soil, which maintains soil structure, can improve soils that have been overfertilized with nitrogen and phosphate. The nettle yields range from 6-15 t/ha, which depends on the level of fertilization, agronomic treatments, soil type, and nettle plant can be used to produce biofertilizer and new high-quality agricultural raw materials for the production of various dyes, for the textile industry and the energy sector (Dreyer, 1996; Hartl & Vogl, 2002; Lehtomäki, 2006; Guil-Guerrero et al., 2014; Dubrovskis et al., 2018; Garmendia et al., 2018).The chemical composition of extracted nettle fibres was 65-85% cellulose, 5-12% hemicellulose, 2-4% lignin (Agus Suryawan et al*.,* 2017; Viotti et al*.,* 2022). The stinging nettle dry biomass contained 45.76% C, 5.60% H, 0.87 % N, 0.10% S, 6.93% ash and 17.48 MJ/ kg NCV, the specific density of pellets reached a maximum of 1068 kg/m^3 (Jankauskiene et al., 2016).

Nettle could be a promising candidate contributing to the reduction of atmospheric GHG emissions, it consumes significantly larger quantities of atmospheric CO_2 (18.8 t/ha), in relation to biomass, than mature forests (Butkute et al., 2015).

The aim of the current study was to evaluate some biological peculiarities, the quality of fresh and ensiled biomass of stinging nettle *Urtica dioica* L.*,* as feed for ruminant animals, as well as substrate for the production of biomethane by anaerobic digestion.

MATERIALS AND METHODS

The localecotype of stinging nettle *Urtica dioica*, which growsin the experimental plot of the National Botanical Garden (Institute) of Moldova, Chişinău, N 46°58′25.7″ latitude and E 28°52′57.8″, served as subject of research, andthe common sainfoin, *Onobrychis viciifolia* '*Anamaria*' and the low-coumarin local ecotype of yellow sweet clover, *Melilotus officinalis*, were used as control variants. The experimental design was a randomised complete block design with four replications, and the experimental plots measured 10 m^2. The plant growth, development and productivity were assessed according to methodical indications. The stinging nettle and yellow clover green mass were harvested in the flowering period, common sainfoin - in the budding-flowering stage. The green mass yield was measured by weighing. The dry matter content was determined by drying the samples up to constant weight at a temperature of 105°C. For ensiling, the harvested green mass was chopped with a stationary forage chopping unit, shredded and compressed in well-sealed containers. After 45 days, the containers were opened and the sensorial and chemical characteristics of the 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 a temperature of 60°C, then they were 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) were determined by the near infrared spectroscopy (NIRS) technique PERTEN DA 7200. The concentration of cellulose (Cel), hemicellulose (HC), digestible energy (DE), metabolizable energy (ME), net energy for lactation (NEl), dry matter digestibility (DMD) and relative feed value (RFV) were calculated according to standard procedures.

The carbon content of the substrates was determined using an empirical equation according to Badger et al. (1979). The biochemical methane potential was calculated according to the equations indicated by Dandikas et al. (2015).

RESULTS AND DISCUSSIONS

In the second year, the local ecotype of *Urtica dioica* started active growth in the first days of March, common sainfoin, *Onobrychis viciifolia* - at the end of March, but yellow sweet clover, *Melilotus officinalis*– in the first days of April. At the time when the green mass was harvested, the *Urtica dioica* plants reached 138 cm in height, *Melilotus officinalis* - 112 cm, but *Onobrychis viciifolia* plants - 99 cm. The productivity of *Urtica dioica* was 4.01 kg/m^2 green mass or 0.83 kg/m^{2}dry matter, *Melilotus officinalis* reached respectively 3.78 kg/m^{2}green mass or 1.17 kg/m^2 dry matter, but the yield of *Onobrychis viciifolia* was 4.23 kg/m^2 green mass or 1.01 kg/m^2 dry matter.

The biochemical composition, nutritive and energy value of the green mass harvested from the tested species are presented in Table 1. Analysing the results of the biochemical composition of green mass, we found that the dry matter of the studied species differs essentially in the concentration of crude protein, crude fibre, cell wall fractions and ash. We found that *Urtica dioica* whole plantswere characterised by very high content of crude protein and optimal content of crude fibre as compared with *Onobrychis viciifolia* and *Melilotus officinalis*. The content of minerals in *Urtica dioica* is higher than in *Onobrychis viciifolia* and lower than in *Melilotus officinalis.* The concentration of neutral detergent fibre and acid detergent lignin is higher than in the control legume species. *Urtica dioica* harvested green mass contained very high amounts of hemicellulose. The dry matter digestibility and energy concentration is lower in *Urtica dioica* mass than in *Onobrychis viciifolia*, but does not differ much from *Melilotus officinalis* green mass.

Table 1. The biochemical composition and the nutritive value of the harvested green mass of the studied species

Indices	*Urtica dioica*	*Onobrychis viciifolia*	*Melilotus officinalis*
Crude protein, g/kg DM	207	177	179
Crude fibre, g/kg DM	268	293	330
Minerals, g/kgDM	106	96	118
Acid detergent fibre, g/kg DM	329	309	331
Neutral detergent fibre, g/kg DM	574	447	473
Acid detergent lignin, g/kg DM	66	49	44
Cellulose, g/kg DM	263	260	287
Hemicellulose, g/kg DM	245	138	142
Dry matter digestibility, g/kg DM	633	648	631
Relative feed value	103	135	124
Digestible energy, MJ/ kg	12.47	12.73	12.42
Metabolizable energy, MJ/ kg	10.23	10.45	10.20
Net energy for lactation, MJ/ kg	6.25	6.48	6.22

The results of the estimation of the quality of green mass from *Urticaceae* species are given in the specialized literature. According to Larin et al. (1952) *Urtica dioica* plants contained in dry matter 15.8-23.3% CP, 2.5-5.0% EE, 13.0-27.1 % CF, 35.0-44.5% NFE. Medvedev & Smetannikova (1981) showed that nettle dry matter contained 23.8% CP, 3.7 % EE, 24.5% CF, 33.1% NFE, 16.4% ash, the nutritive value was 0.19 feed unit/kg green fodder. Bogachkov & Morozov (1990) found that nettles contained in the dry matter 19.7% CP, 2.27% EE, 40.03% NFE, 24.4% CF, 14.39% ash, 3.32% Ca and 0.31% P.Nielsen & Soegaard (2000) remarked that the forage quality of *Urtica dioica* plants grown in semi-natural grassland, cut in June-July, was 318-364 g/kg NDF and 674-748 g/kg IVOMD, but in *Trifolium pratense* forage, it was 361-440.4 g/kg NDF and 570-673 g/kg IVOMD. Kshnikatkina et al. (2005) reported that the dry matter from *Urtica dioica* contained 20.73% CP, 3.72% EE, 14.18% CF, 40.30% NFE, 14.19% ash, 1.70% Ca, 0.80% P. Biesiada et al. (2010) remarked that the content of crude protein in nettle leaves was 17.31-24.12% in first cut mass, 19.37-24.50% in second cut mass and 21.43-25.68% in third cut mass. Egorov (2014) reported the chemical composition of flour from *Urtica dioica* was 908 g/kg DM, 24.8% CP, 5% EE, 13% CF, 32.91% NFE, 11% sugars, 4.5% starch, 19.1% ash, 1.4% Ca and 0.5% P. Guil-Guerrero et al. (2014) compared the quality of green mass from *Urtica* species and found that the chemical composition and energy value of *Urtica dioica* was 33.3% CP, 1.1% EE, 11.1% CF, 21.1% ash, 33.3% carbohydrates and 15.67 MJ/kg GE, but *Urtica urens* - 20.0% CP, 1.7% EE, 15.3% CF, 14.6% ash, 51.7% carbohydrates and 16.20 MJ/kg GE, respectively. Adhikari et al. (2015) showed that *Urtica dioica* flour contained 33.8% CP, 9.1% CF, 3.6% EE, 16.2% ash, 37.4% carbohydrates and 3070 kcal/kg energy value. Ciopata et al. (2015) reported that the chemical content of *Urtica dioica* was 27.8% CP, 15.3% CF, 28.4% NDF, 19.5% ADF, 20.72% ash and 73.8% ODM, but *Trifolium repens* 28.7% CP, 17.9% CF, 30.9% NDF, 22.8% ADF, 10.99% ash, 72.8% ODM. Kulivand & Kafilzadeh (2015) remarked that *Urtica dioica* contained 11.95% CP, 1.45%EE, 49.52% NDF, 39.06% ADF, 18.2% ash. Alieva et al. (2016) showed that the nutrient content in *Urtica dioica* plants was 22.88-23.15% CP, 2.12-2.81% EE, 12.47-13.00% CF, 32.91-35.99% NFE and 17.01-19.10% ash. Andualem et al. (2016) studied the biochemical composition of *Urtica simensis* whole plants and found that the fresh mass contained 167 g/kg dry matter, 25.4% CP, 39.3% NDFom, 21.4% ADFom, 4.29% ADL, 21.7% ash. Yakovchik & Yakovchik, (2017) reported that *Urtica dioica* contained 22-23% CP, 18-21% CF,75-85% dry matter digestibility, 70-75 g/kg carotene, 0.18-0.19 nutritive units/kg green mass and 190-210 g DP/nutritive units, but *Urtica cannabina* 21-22 % CP, 2.5-3.0% EE, 20-22% CF, 7-9% ash. Arros et al. (2019) mentionedthat the chemical and mineral composition of *Urticaurens* leaf powder was 24% CP, 2.5-5.0% EE, 8.7% CF, 31.6% NFE, 29.1% ash, 1.65% Ca, 0.51% P, 0.44% Mg. Zhang et al. (2020, 2022) remarked that the composition and nutritive value of *Urtica cannabina* was 16.5% CP, 2.9%EE, 38.2% NDF, 30.1% ADF, 29.5% NDC, 3.81% lignin, 18.9% ash, 3.44% Ca, 1.6% P and 58.4% IVDMD. Kosolapov et al. (2022) noted that *Urtica dioica* fodder contained 21.1-24.2% CP, 2.5-4.2% EE, 12.0-14.8% CF, 15.0% - 17.6% ash. Huang et al. (2023) indicated that whole plants of *Urtica cannabina* contained 310.9 g/kg DM, 12.57% CP and 6.18% WSC.

Table 2. The biochemical composition and the nutritive value of the ensiled mass from the studied species

Indices	*Urtica dioica*	*Onobrychis viciifolia*	*Melilotus officinalis*
Crude protein, g/kg DM	221	142	178
Crude fibre, g/kg DM	283	312	348
Minerals, g/kg DM	145	118	103
Acid detergent fibre, g/kg DM	338	317	333
Neutral detergent fibre, g/kg DM	530	470	462
Acid detergent lignin, g/kg DM	65	40	38
Cellulose, g/kg DM	273	277	285
Hemicellulose, g/kg DM	192	153	129
Dry matter digestibility, g/kg DM	626	642	630
Relative feed value	110	127	129
Digestible energy, MJ/kg DM	12.34	12.63	12.41
Metabolizable energy, MJ/kg DM	10.13	10.37	10.19
Net energy for lactation, MJ/kg DM	6.14	6.38	6.20

The proportion of conserved forages significantly increased in relation to the total yearly feed production, and the feed quality has markedly improved during the last 50 years. During times of plentiful growth, fodders can be stored as silage or hay. Currently, silage is the most common source of preserved feed for ruminant animals.

When opening the glass containers with prepared silage from the studied species, there was no gas or juice leakage from the preserved mass. The analysed stinging nettle *Urtica* *dioica* silage was characterized by dark green leaves and yellow stems, specific smell, the consistency was preserved in comparison with the initial green mass, without mould and mucus. As a result of the performed analysis, it was determined that the fermentation profile of stinging nettle silage was characterized by: pH=7.60, 32.2 g/kg total organic acids, 7.5 g/kg free lactic acid, 0.6 g/kg free acetic acid, 6.8 g/kg fixed lactic acid, 5.9 g/kg fixed acetic acid, 12.4 g/kg fixed butyric acid.

Analysing the biochemical composition of ensiled mass from the studied species, Table 2, it has been determined that the concentrations of nutrients in the dry matter varied as follows: 142-221 g/kg CP, 283-348 g/kg CF, 317-338 g/kg ADF, 462-530 g/kg NDF, 38-65 g/kg ADL, 273-285 g/kg Cel, 129-192g/kg HC and 103-145 g/kg ash. The nutritive and energy values of the ensiled mass were 62.6-64.2 % DMD, RFV=110-129, 12.34-12.63 MJ/kg DE, 10.13-10.19 MJ/kg ME and 6.14-6.20 MJ/kg NEl. As compared with the harvested mass, the silage from *Urtica dioica* had high concentration of crude protein, crude fibre, minerals, and low content of neutral detergent fibre and hemicellulose. We would like to mention that the ensiled mass from stinging nettle, as compared with the studied leguminous species, is characterized by higher content of de crude protein, crude fibre,cell wall fractions (NDF, ADF, ADL), hemicellulose and low cellulose nutritional value and energy supply of the feed. The nutritional value and energy supply of the ensiled stinging nettle feed is similar to that of sweet clover silage, but lower than common sainfoin haylage.

In the literature sources, there is little information regarding the chemical composition and nutritional value of ensiled mass from *Urtica* species. According to Medvedev & Smetannikova (1981), *Urtica cannabina* silage contained 33% DM, including 5.1% CP, 0.1% EE, 4.8% CF, 17.5% NFE, 5.5% ash, 22 g/kg DP and 0.175feed unit/kg, but rapeseed silage - 12.7% DM, including 2.4% CP, 0.1% EE, 1.3% CF, 5.0% NFE, 2.7% ash, 37 g/kg DP and 0.1feed unit/kg silage. Zhang et al. (2014) reported that *Urtica cannabina* silage provides more than 200 g/kg DM crude protein and had 74 % dry matter digestibility. A study conducted by Zhang and co-workers (2015) showed that the quality of pure *Urtica cannabina* silage was 209 g/kg DM, 19.0 % CP, 34.7% NDF, 28.0% ADF, 1.6% EE, 1.85% WSC, 57.0% IVDMD, 34.5% IVNDF, 32.9% IVADF, mixed silage with corn flour 316 g/kg DM, 16.8% CP, 27.6% NDF, 24.9% ADF, 1.45% EE, 2.15% WSC, 77.8% IVDMD, 46.8% IVNDF, 42.4% IVADF. Huang et al. (2023) found that *Urtica cannabina* silage is characterized by 294.7-303.6 g/kg DM, 11.52-11.65% CP, 4.96-4.81% WSC, pH=6.29-6.34, 28.0-29.7 g/kg lactic acid, 6.2-7.4 g/kg acetic acid, 4.5-5.7 g/kg propionic acid, 5.49-5.67% ammonium-nitrogen.

The depletion of fossil fuels, environmental pollution and energy insecurity have become global challenges in recent years. Renewable energy sources coming from biomass could play an important role in terms of energy supply and positive environmental effects. Biogas has become important as a renewable source of energy because of its decentralized approach, and it can be used to obtain heat and electrical power in special installations, but also as fuel in internal combustion engines. Many bacteria affect anaerobic digestion, including acetic acid-forming bacteria (acetogens) and methane-forming bacteria (methanogens). These microorganisms are very sensitive to environmental variations since they are obligatory anaerobic. These organisms promote a number of chemical processes in converting the biomass to biogas. The carbon to nitrogen ratio constitutes a basic factor governing the correct course of methane fermentation.

The results regarding the biochemical biomethane production potential of investigated substrates are shown in Table 3. The nitrogen concentration in the tested *Urtica dioica* substrates ranged from 33.12 g/kg to 35.36 g/kg, the estimated content of carbon - from 475.00 g/kg to 496.67 g/kg, the C/N = 13-15, but the substrates from the studied leguminous species contained 22.72-28.64 g/kg nitrogen, 490.00-502.22 g/kg carbon and C/N = 18-22. Essential differences were observed between concentrations of hemicellulose and acid detergent lignin. The *Urtica dioica* substrates contained high concentration of these substances. The biochemical methane potential of the tested stinging nettle substrates did not vary essentially - 319-321 l/kg VS, but was lower in comparison with the substrates of yellow sweet clover and common sainfoin.

Table 3. The biochemical biomethane production potential of the researched substrates

Indices	*Urtica dioica*		*Onobrychis viciifolia*		*Melilotus officinalis*	
	green mass	silage	green mass	haylage	green mass	silage
Crude protein, g/kg DM	207.00	221.00	177.00	142.00	179.00	178.00
Minerals, g/kg DM	106.00	145.00	96.00	118.00	118.00	103.00
Nitrogen, g/kg DM	33.12	35.36	28.32	22.72	28.64	28.40
Carbon, g/kg DM	496.67	475.00	502.22	490.00	490.00	498.33
Ratio carbon/nitrogen	15	13	18	22	17	18
Hemicellulose, g/kg DM	245.00	192.00	138.00	153.00	142.00	129.00
Acid detergent lignin, g/kg DM	66.00	65.00	49.00	40.00	44.00	38.00
Biomethane potential, L/kg VS	319	321	340	343	344	353

Several publications have documented the biomethane production potential of substrates from *Urtica* species. According to Lehtomaki (2006) the methane potential of common nettle achieved3000-5000 m^3/ha/year or 30-50 MWh/ha/year. Lehtomäki et al., 2008, remarked that harvested nettle biomass contained 150-303 g/kg DM, 123-283 g/kg OM, 12.0-42.0 g/kg nitrogen, 410-472 g/kg carbon, C/N=10-41, 189-280 g/kg lignin, the specific methane yield varied from 210 to 420 l/kg VS, but red cloverbiomass contained 153-399 g/kg DM, 138-387 g/kg OM, 19.0-52.0 g/kg nitrogen, 449-478 g/kg carbon, C/N = 9-25, 185-224 g/kg lign in with specific methane yield 280-300 l/kg VS. Wellinger et al. (2013) reported that the dry matter yield of nettles was 6-10 t/ha, the methane yield 2200-3600 m^3/ha/year and energy 21-35 MWh/ha/year. Dubrovskis et al. (2018) found that the average specific biogas or methane production per unit of dry organic matter added (DOM) from common nettle was 0.709 L/g or 0.324 L/g, but from common nettle with biocatalyst Metaferm - 0.752 L/g or 0.328 L/g, respectively. Cepo (2021) mentioned that the biogas potential of *Urtica dioica* substrate was 0.43 m^3/kg of dry organic matter and methane potential 0.25 m^3/kg of dry organic matter.

CONCLUSIONS

The dry matter of the local ecotype of common nettle, *Urtica dioica* whole plants harvested in the flowering period contained 20.7% CP, 10.6% ash, 26.8% CF, 32.9% ADF, 57.4% NDF, 6.6% ADL, 26.3% Cel, 24.5% HC, with forage value 633 g/kg DDM, RFV = 103, 12.47 MJ/kg DE, 10.23 MJ/kg ME and 6.25 MJ/kg NEl.

The ensiled mass contained 22.1% CP, 14.5% ash, 28.3%CF, 33.8% ADF, 53.0% NDF, 6.6% ADL, 27.3% Cel, 19.2% HC, with forage value 626 g/kg DDM, RFV = 110, 12.34 MJ/kg DE, 10.13 MJ/kg ME and 6.14 MJ/kg NEl.

The studied common nettle fresh and ensiled mass substrates have C/N = 13.4-15.0 and the biochemical methane potential reaches 319-321 l/kg ODM.

The local ecotype of common nettle, *Urtica dioica*, can be used as an alternative forage source for farm animals or as co-substrate in biogas generators for renewable energy production.

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OIL CONTENT OF SUNFLOWER GRAINS ACCORDING TO ROW SPACING AND PLANT DENSITY

Victorița MARIN[1, 2], Lenuța Iuliana EPURE[1], Viorel ION[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, 011464, Bucharest, Romania
[2]State Institute for Variety Testing and Registration, 61 Marasti Blvd, District 1, 011464, Bucharest, Romania

Corresponding author email: victorita_chiriac@istis.ro

Abstract

Sunflower is a major oil crop which produces edible oil with a great importance in human nutrition. The oil content of the sunflower grains is determined genetically but it is influenced by several environmental and technological factors. The technological factors can be controlled by the farmers in view to get higher oil content in the grains, and therefore their influence is important to be known. Starting from this idea, the aim of this paper is to identify the effect of row spacing and plant density on the oil content of the sunflower grains under specific growing conditions of Romania. Research was performed in 2019, 2020, and 2021 in field experiments under rainfed conditions located in four locations, among which one in East Romania, one in South-East Romania and two in South Romania. The experimental factors were the following: Factor A - row spacing, with 3 graduations: a1 = 70 cm; a2 = 60 cm; a3 = 50 cm; Factor B - plant density, with 3 graduations: b1 = 50,000 plants/ha; b2 = 60,000 plants/ha; b3 = 70,000 plants/ha. The obtained data in the specific growing conditions of Romania showed that row spacing and plant density have a small influence on the oil content of the sunflower seeds. Among the two technological parameters, row spacing determined the smallest influence on oil content of the sunflower grains. Increasing of plant densities determined a slight decreasing of the oil content of the sunflower grains. The differences in oil content determined by the row spacing and plant density are not statistically significant in conditions of good water supply of sunflower plants, especially in the period of grain development.

Key words: *sunflower, oil content, row spacing, plant density.*

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the major oil crops which produce edible oil with a great importance in human nutrition at global scale. Sunflower oil is mainly used for human consumption, but also as raw material for the processing industry, as well as for biodiesel production (Mijić et al., 2009; Zheljazkov et al., 2008). The physicochemical properties support the suitability of the sunflower oil for consumption rather than industrial application of soap making (Abitogun et al., 2008). At world level, the sunflower oil is used about 90% for human consumption, and the rest of about 10% for industrial purposes or biodiesel production (Jodić at al., 2015).

The achievement of high grain and oil yields has been the main goal of sunflower production and breeding (Marinković, 1992; Pereyra-Irujo & Aguirrezábal, 2007). Oil content is one of the important yield traits in sunflower (Evci et al., 2012). The oil yield is conditioned by the grain oil content, which is a complex characteristic affected by different factors that may act individually or collectively (Marinković, 1992). The oil content in sunflower grains is determined by the contribution of genotype, environment and the crop management (Cojocaru et al., 2023).

For successful production of edible oil, it is necessary to have sunflower hybrids which are capable of providing high grain yields as well as high oil yields (Petcu et al., 2010), and therefor increasing of oil yield is one of the most important goals in sunflower breeding programs (Mijic et al., 2009). The variation limits of the grain oil content are between 40 and 54% for the present sunflower hybrids cultivated for oil production (Ion, 2021).

In the crop technology, plant population and its arrangement are important aspects of sunflower production that are directly controlled by the farmer (Robinson et al., 1982). Plant population based on row and plant spacing is a major part of agronomic practices (Beg et al., 2007), this having to be correlated with the specific growing conditions. As an example, populations of 60,000 to 75,000 plants/ha at row spacing of 35-60 cm are recommended for sunflower production under dryland conditions (Vijayalakshmi et al., 1975).

Most studies report that oil content is only little affected by plant density variations, plant density contribution to oil content being 3 times less than genotype effect and 2 times less than soil effect (Andrianasolo et al., 2012).

Sunflower crop can be grown over different row spacing which is determining the shape of the nutritional space for a given plant population (Ion et al., 2018). Seed oil content is influenced by row spacing but differences are not biologically important (Rauf et al., 2012).

The aim of this paper is to identify the effect of row spacing and plant density on the oil content of the sunflower grains under specific growing conditions of Romania.

MATERIALS AND METHODS

Research was performed in 2019, 2020, and 2021 in field experiments under rainfed conditions located in four locations in Romania, among which one in East Romania, one in South-East Romania and two in South Romania.

The four locations are the following:
- Negrești, located in eastern part of Romania, in Vaslui County.
- Cogealac, located in southeast part of Romania, in Constanța County.
- Dâlga, located in southern part of Romania, in Călărași County.
- Troian, located in southern part of Romania, in Teleorman County.

The field experiments were organised as subdivided plots with 3 replications. The experimental factors were the following:
- Factor A - row spacing, with 3 graduations:
  - a1 = 70 cm;
  - a2 = 60 cm;
  - a3 = 50 cm.
- Factor B - plant density, with 3 graduations:
  - b1 = 50,000 plants/ha;
  - b2 = 60,000 plants/ha;
  - b3 = 70,000 plants/ha.

Research was conducted for four sunflower hybrids, respectively: KWS Acer (early Clearfield hybrid); NK Neoma (mid-early Clearfield hybrid); P64LE25 (mid-early sulfonylurea resistant hybrid); Subaro (mid-late sulfonylurea resistant hybrid). The data presented in the present paper are the average values for the four studied sunflower hybrids.

The preceding crop was winter wheat in all locations and experimental years. Fertilisation consisted of applying 40-60 kg/ha of nitrogen and 40-60 kg/ha of phosphorus by spreading 200-300 kg/ha of 20:20:0 complex fertiliser before seedbed preparation, according to soil conditions in each location.

Tillage consisted in ploughing in autumn, one disk harrow passage in March, and seedbed preparation before sowing.

Sowing was performed in the first two decades of April except for Troian location where sowing was performed either at the end of April or at beginning of May, and Dâlga location in 2021, when sowing was performed at beginning of May (Table 1).

Table 1. Sowing data according to location and year

Location	Year		
	2019	2020	2021
Negrești	09 of April	06 of April	19 of April
Cogealac	11 of April	10 of April	16 of April
Dâlga	10 of April	14 of April	03 of May
Troian	03 of May	29 of April	05 of May

For weed control, herbicide Dual Gold 960 EC (S-metolachlor 960 g/l) was applied in a rate of 1.5 l/ha either before seedbed preparation or after sowing, but before emergence. Also, for controlling the monocotyledonous weeds, the herbicide Select Super (Clethodim 120 g/l) was applied in the growth period of sunflower plants in a rate of 0.8-1.3 l/ha, according to the weeds species (annual or perennial) identified in the experimental field.

The sunflower heads of each experimental variant were harvested in the stage of full maturity. The grain oil content was determined by the help of a NMR analyser.

The data were statistically processed by the analysis of variance (ANOVA). Also, the correlation coefficients were calculated.

In all locations of research, the year 2020 was the warmest, while the year 2019 was the coldest except for Negresti location where the coldest year was 2021 (Table 2). Among the four locations, the highest average temperatures were registered in Cogealac and Dâlga, while the smallest average temperatures were registered in Troian location. Regardless of location and year, the registered temperatures were higher than the multiannual average specific for each location.

In all locations of research, the year 2021 was the rainiest year, while the warmest year 2020 was the driest (Table 2). Among the four locations, the Negrești and Troian locations were the wettest and the Cogealac and Dâlga were the driest. Dâlga location was exposed to extreme rainfall, with a very dry year 2020 (340.9 mm rainfall) and a very wet year 2021 (851.7 mm rainfall).

The soil was of chernozem type in all locations except for Negrești location where the soil is of cambic chernozem type.

Table 2. Temperatures and rainfall in the four locations and the three experimental years

Location	2019*	2020	2021	Multiannual average
Temperatures (°C)				
Negrești	12.9	12.2	12.1	9.5
Cogealac	13.3	14.3	13.4	10.7
Dâlga	13.1	14.2	13.8	11.0
Troian	11.1	12.1	11.5	10.4
Rainfall (mm)				
Negrești	539.8	416.1	634.2	420
Cogealac	362.0	340.0	580.0	352
Dâlga	476.5	340.9	851.7	503.6
Troian	535.0	488.0	639.0	550.1

*The climatic data of the experimental years are presented for the period between September of the previous year to August of the current year, when the sunflower vegetation period generally ends or approach the end for the specific growing conditions in Romania.

RESULTS AND DISCUSSIONS

In the year 2019, compared to control variant (row spacing of 70 cm and plant density of 50,000 plants/ha), in two of the four locations, respectively in Negrești and Dâlga locations, there were registered negative differences generally statistically significant (Table 3). In the other two locations (Cogealac and Troian locations) the differences were mostly negative but there are not statistically significant (except two variants in Cogealac location). This means that generally decreasing the row spacing and increasing the plant density were associated with a decrease of the oil content in the sunflower seeds.

In the year 2020, the warmest and the driest year in all locations of research, the differences compared to control variant were not statistically significant in two locations (Negrești and Troian locations), except two variants in Troian location (Table 4). In Dâlga location, the differences registered at higher plant densities were negative and statistically significant, especially at 70,000 plants/ha and row spacing of 60 and 70 cm. In Cogealac location, where there were registered the highest temperatures and the smallest rainfall in 2020, the differences compared to control variant were positive and statistically significant at two variants (row spacing of 70 cm and plant population of 70,000 plants/ha, and row spacing of 50 cm and 50,000 plants/ha).

In the year 2021, the rainiest year in all locations of research, except two experimental variants (row spacing of 50 cm and plant density of 50,000 plants/ha at Negrești location, and row spacing of 50 cm and plant density of 70,000 plants/ha at Cogealac location), the differences registered compared to control variants were not statistically significant (Table 5).

Table 3. Oil content of sunflower grains according to row spacing and plant density in different locations in 2019

Experimental factors		Negrești location		Cogealac location		Dâlga location		Troian location	
Row spacing (cm)	Plant density (plants/ha)	Oil content (%)	Difference to control (%)	Oil content (%)	Difference to control (%)	Oil content (%)	Difference to control (%)	Oil content (%)	Difference to control (%)
70	50,000	44.40	Control	44.25	Control	44.48	Control	44.22	Control
	60,000	44.28	-0.12	43.98	-0.27 ^{o}	44.02	-0.46 ^{ooo}	44.03	-0.19
	70,000	43.85	-0.55 ^{ooo}	44.35	0.10	44.40	-0.08	44.20	-0.02
60	50,000	44.38	-0.02	44.02	-0.23	44.13	-0.35 ^{ooo}	44.30	0.08
	60,000	44.13	-0.27 ^{oo}	44.02	-0.23	44.17	-0.31 ^{oo}	44.18	-0.04
	70,000	43.92	-0.48 ^{ooo}	44.35	0.10	44.02	-0.46 ^{ooo}	44.02	-0.20
50	50,000	44.30	-0.10	44.38	0.13	44.28	-0.20 ^{o}	44.15	-0.07
	60,000	43.90	-0.50 ^{ooo}	44.30	0.05	43.95	-0.53 ^{ooo}	44.08	-0.14
	70,000	44.38	-0.02	43.98	-0.27 ^{o}	44.48	0	44.42	0.2
	DL5%		*0.169*		*0.235*		*0.190*		*0.212*
	DL1%		*0.228*		*0.317*		*0.256*		*0.286*
	DL0.1%		*0.304*		*0.422*		*0.342*		*0.381*

Table 4. Oil content of sunflower grains according to row spacing and plant density in different locations in 2020

Experimental factors		Negrești location		Cogealac location		Dâlga location		Troian location	
Row spacing (cm)	Plant density (plants/ha)	Oil content (%)	Difference to control (%)	Oil content (%)	Difference to control (%)	Oil content (%)	Difference to control (%)	Oil content (%)	Difference to control (%)
70	50,000	44.17	Control	44.30	Control	44.52	Control	44.00	Control
	60,000	44.07	-0.10	44.27	-0.03	44.42	-0.10	44.00	0
	70,000	44.00	-0.17	44.50	0.20 *	44.25	-0.27 ^{oo}	43.50	-0.50 ^{o}
60	50,000	44.25	0.08	44.20	-0.10	44.43	-0.09	44.00	0
	60,000	44.28	0.11	44.40	0.10	44.43	-0.09	44.00	0
	70,000	44.08	-0.09	44.45	0.15	44.20	-0.32 ^{ooo}	43.50	-0.50 ^{o}
50	50,000	44.32	0.15	44.60	0.30 **	44.53	0.01	44.00	0
	60,000	44.33	0.16	44.30	0	44.22	-0.30 ^{oo}	44.00	0
	70,000	44.35	0.18	44.10	-0.20 ^{o}	44.42	-0.10	44.00	0
	DL5%		*0.304*		*0.172*		*0.177*		*0.395*
	DL1%		*0.411*		*0.232*		*0.239*		*0.533*
	DL0.1%		*0.547*		*0.309*		*0.319*		*0.710*

Table 5. Oil content of sunflower grains according to row spacing and plant density in different locations in 2021

Experimental factors		Negrești location		Cogealac location		Dâlga location		Troian location	
Row spacing (cm)	Plant density (plants/ha)	Oil content (%)	Difference to control (%)	Oil content (%)	Difference to control (%)	Oil content (%)	Difference to control (%)	Oil content (%)	Difference to control (%)
70	50,000	43.88	Control	44.17	Control	44.30	Control	44.30	Control
	60,000	44.05	0.17	44.32	0.15	44.20	-0.1	44.18	-0.12
	70,000	44.20	0.32	44.50	0.33	44.07	-0.23	44.15	-0.15
60	50,000	44.05	0.17	44.12	-0.05	44.33	0.03	44.38	0.08
	60,000	44.17	0.29	44.38	0.21	44.43	0.13	44.35	0.05
	70,000	44.08	0.20	44.10	-0.07	44.10	-0.2	44.15	-0.15
50	50,000	44.38	0.50 *	44.22	0.05	44.13	-0.17	44.27	-0.03
	60,000	44.25	0.37	44.30	0.13	44.10	-0.2	44.27	-0.03
	70,000	43.98	0.10	43.72	-0.45 ^{o}	44.35	0.05	44.48	0.18
	DL5%		*0,405*		*0.358*		*0.391*		*0.191*
	DL1%		*0,547*		*0.484*		*0.528*		*0.258*
	DL0.1%		*0,729*		*0.644*		*0.703*		*0.343*

As average values in all locations and for all experimental years, the smallest oil content was registered at row spacing of 60 cm (44.19%), while the highest oil content was registered at row spacing of 70 cm (44.21%) (Figure 1). Anyway, the differences between values registered at different row spacing were quite small. In contrast to the row spacing, the plant density determined more evident differences of the oil content, the increasing of plant densities determining the decreasing of the oil content. However, it has to be underlined that there is a slight difference of 0.1% between 44.26% registered at plant density of 50.000 plants/ha to 44.16% registered at plant density of 70.000 plants/ha.

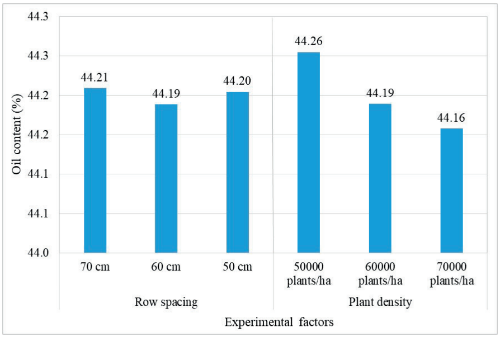

Figure 1. Oil content of sunflower seeds as average values according to row spacing and plants density

As research was conducted through three years, it was calculated the correlation coefficient of the oil content of the sunflower grains with the monthly average temperature during the vegetation period as well as with the monthly rainfall sum (Table 6).

The oil content correlates positively with the average monthly temperatures but the correlations are very weak. However, the oil content best correlates with the average monthly temperature in June and July when the grains develop (generally, the grains start to develop by the end of June and they are in fully development stage in July for the growing conditions of Romania).

As concerning the correlations of the oil content with the monthly rainfall sum, except July, there are negative correlations, the most important being the negative reasonable correlation of oil content with the rainfall sum of May month. In July, when the sunflower grains are in the development stage in the growing conditions of Romania, there is a weak but positive correlation of the oil content with the rainfall sum.

Table 6. Correlation coefficient of oil content of sunflower grains with average monthly temperatures and monthly rainfall sum

Month	Correlation coefficient of oil content of sunflower grains with:	
	Average temperature (ºC)	Rainfall sum (mm)
April	0.084381	-0.13252
May	0.144245	-0.56124
June	0.18849	-0.13863
July	0.119515	0.108604
August	0.021573	-0.02886

CONCLUSIONS

The obtained data in the specific growing conditions of Romania showed that row spacing and plant density have a small influence on the oil content of the sunflower seeds. Among the two technological parameters, row spacing determined the smallest influence on oil content of the sunflower grains. As concerning plant density, the increasing of plant densities determined a slight decreasing of the oil content of the sunflower grains. The differences in oil content determined by the row spacing and plant density are not statistically significant in conditions of good water supply of sunflower plants, especially in the period of grain development.

The oil content correlates positively with the average monthly temperatures during the vegetation period of the sunflower plants, but the correlations are very weak, the best correlations being with the average monthly temperature in June and July when the grains start to develop and respectively they develop.

The oil content correlates negatively with the monthly rainfall sum during the vegetation period of the sunflower plants, except in July when the sunflower seeds are in the development stage, when there is a very weak positively correlation.

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CROP ROTATION IMPACT ON THE MAIZE CROP PESTS

Alexandru RANTEȘ

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, 011464, Bucharest, Romania

Corresponding author email: alex_rantes@yahoo.com

Abstract

Due to its high yielding capacity and its adaptability, the maize crop has become the main crop grown by Romanian farmers. Although the most important problem in maize cultivation were weed control, in recent years the pest control has become as important as the weed control. Due to the long monoculture, farmers are faced with very high pest densities, which leads to a reduction in yield but also in its quality, in extreme cases completely compromising the crop, making it necessary to reseed the crop. Pests of the maize crop target its entire vegetation period, and therefore farmers are subject to higher production costs by applying additional insecticide treatments. The objective of the present paper is to present the influence of crop rotation on the density and the attack intensity of the main pest of the maize crop. The research was performed under field conditions in South Romania (Giurgiu county, Putineiu location) in 2022, and the pests taken into account were Tanymecus dilaticollis Gyll. (maize leaf weevil), Helicoverpa armigera Hb. (cotton bollworm or corn earworm) and Ostrinia nubilalis Hbn. (corn borer).

Key words: *maize, Tanymecus dilaticollis, Helicoverpa armigera, Ostrinia nubilalis, crop rotation.*

INTRODUCTION

Maize crop is the main crop grown by Romanian farmers. As a C4 plant, maize has a high capacity to produce biomass (Dicu et al., 2016), and it has the highest grain yield potential among the cereal crops (Ion et al., 2015b).

Crop rotation is used by farmers for centuries with the purpose of preventing crop diseases and pests, as well as nutrient imbalances in the soil, all these to make production more efficient and to obtain qualitative yields.

The crop rotation is the practice of alternating the annual crops grown in a specific field in a planned pattern or sequence so that the crops of a same species or family are not grown repeatedly without interruption on the same field. Crop rotation is a critical feature of all organic cropping systems because it provides the principal mechanism for building healthy soils, a major way to control pests, and a variety of other benefits. Crop rotation means changing the type of crop grown on a particular piece of land from year to year (Charles Mohler, 2009).

Crop rotation used to be the driving means of controlling pests and weeds for thousands of years, before the green revolution, however today various management practices exist for this purpose. (European Commission DG ENV, 2010).

Growing the same crop on the same agricultural area for many consecutive years is known as monoculture and it gradually depletes the soil of certain nutrients and greatly favors the multiplication of specific diseases, pests and weeds (Roman, 2011).

The preceding crop is an important crop technology measure with a significant influence upon the yield (Ion et al., 2015a).

Crop rotation should be based on some criteria, such as vegetation period of cultivated plants, their water and nutrients requirements, as well as their specific diseases and pests, or the plant capacity to leave nutrients in the soil, such as leguminous plants etc.

The benefits of crop rotation for land and water resource protection and productivity have been identified, but many of the rotation factors, processes and mechanisms responsible for increased yield and other benefits need to be better understood (Berzsenyi et al., 2000).

The effect of crop rotation on maize yield is inversely proportional to the ratio of the maize in the crop rotation (Šeremešić et al., 2013). This effect has represented and continues to do so, the object of many research performed

under different soil and climatic conditions (Ștefan et al., 2018).
Pests are most easily kept in balance when different crops are grown over a number of years. Rotate susceptible crops at intervals to inhibit the buildup of their specific pest organisms. Rotation length should be based on the amount of time soil-borne pathogens remain viable in the field (Tennessee Department of Agriculture).
Maize leaf weevil (*Tanymecus dilaticollis* Gyll.) is considered to be a regional pest in the European Union, been located mainly in the south-east countries (Meislle et al., 2010). In Romania, this is one of the most dangerous pests of the maize crops, about one million hectares cultivated with maize being attacked each year by this pest with different levels of attack intensities (Georgescu et al., 2011).
The corn earworm (*Helicoverpa armigera* Hb.) is a cosmopolitan, widespread species (Pălăgesiu and Crista, 2010), but this is beginning to become a growing problem for European maize producers and implicitly for those from Romania (Grozea et al., 2019).
Ostrinia nubilalis Hbn. is spread throughout Romania, the frequency of attack is, on average, between 30.3% and 70%, larvae causing attacks and production losses by feeding with different parts of the maize plant (stem, cobs, inflorescence) (Pintilie et al., 2022).
The objective of the present paper is to present the influence of crop rotation on the density and the attack intensity of the main pest of the maize crop.

MATERIALS AND METHODS

The research was conducted in experimental plots located in Southern area of Romania, 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 2022.
The climate of the year 2022 was favorable for maize growing. At the time of sowing, the soil had both moisture and temperature sufficient for a good germination of the seeds (Figures 1 and 2).

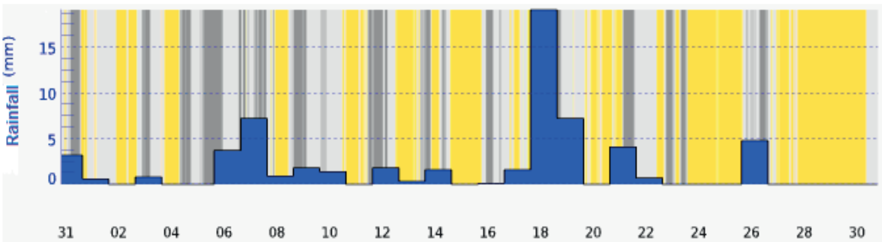

Figure 1. The amount of rainfall in April 2022 (source: https://www.meteoblue.com/)

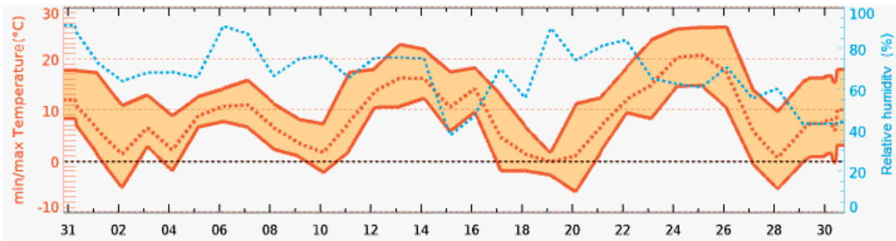

Figure 2. Temperatures recorded in April 2022 (source: https://www.meteoblue.com/)

The experimental plots were sown on April 10th. The cultivation technology was a classic one for maize cultivation, with deep plowing in the fall, followed in the spring by fertilizing the land, incorporating fertilizers into the soil with a cultivator and then sowing.
The preceding crops were the following: winter wheat; peas; soybean; rapeseed; maize monoculture in year 1; maize monoculture in year 2; maize monoculture in year 3.
The seeds were treated with Nuprid AL 600 FS (imidacloprid 600 g/l) with the rate of 8 l/t.

In the experimental plots, the pest density and the intensity of the pest attack on the maize crop were monitored depending on the preceding plant. The main pests that constituted the subject of this experiment were: *Tanymecus dilaticollis* Gyll. (maize leaf weevil), *Helicoverpa armigera* (Hübner) (cotton bollworm or corn earworm) and *Ostrinia nubilalis* (Hübner) (corn borer).
The attack intensity of *T. dilaticollis* was measured with the help of Paulian's scale.
According to this grading scale, the intensity of the attack ranges from 1 (plant not attacked) to 9 (completely destroyed plant).
Pheromonal traps were used to measure the pest density of *H. armigera* and *O. nubilalis*.
The intensity of the attack by *H. armigera* and *O. nubilalis* was determined by consecutively counting on 100 maize plants and observing how many of them were attacked, thus resulting in the attack percentage.

RESULTS AND DISCUSSIONS

The pest density in the experimental plots for *Tanymecus dilaticollis* registered the highest values in the case of maize monoculture, especially when the monoculture was performed for 2 and 3 years (Figure 3). This is according to the findings of Georgescu et al. (2011) who identified in south-east of Romania that maize monoculture has an increasing effect on pest density associate with a higher impact on the attack. It is interesting to highlight that the pest density in the case of wheat as preceding crop for maize was comparable with those obtain when the maize followed maize as preceding crop for one year. The smallest values were registered when maize followed peas and rapeseed.
The pest density in the experimental plots for *Helicoverpa armigera* registered the highest values in the case of maize monoculture, especially when the monoculture was performed for 2 and 3 years (Figure 4). In the case of this pest, it is interesting to highlight that the density in the case of soybean as preceding crop for maize was quite high, even higher than values registered when the maize followed maize for one year. The smallest values were registered when maize followed, wheat, peas and rapeseed.

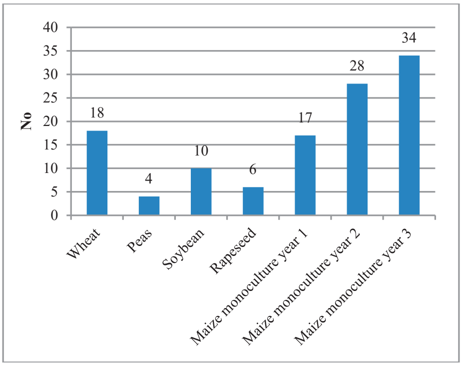

Figure 3. Pest density (number/m^2) for *T. dilaticollis*

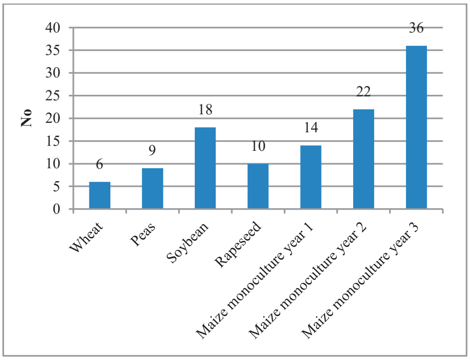

Figure 4. Pest density (number/trap) for *H. armigera*

As in the case of the preceding pests, the pest density in the experimental plots for *Ostrinia nubilalis* registered the highest values in the case of maize monoculture, especially when the monoculture was performed for 3 years (Figure 5). When the maize followed other crops (peas, soybean, wheat and rapeseed) than maize, the registered values were small.

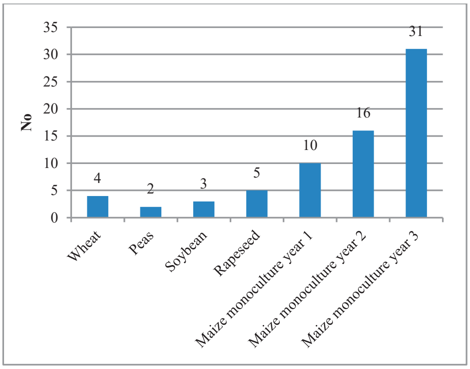

Figure 5. Pest density (number/trap) for *O. nubilalis*

The attack intensity of the *T. dilaticollis* registered the highest grade in the 3-year maize monoculture (Figure 6). The lowest grade was recorded for the peas as preceding crop.

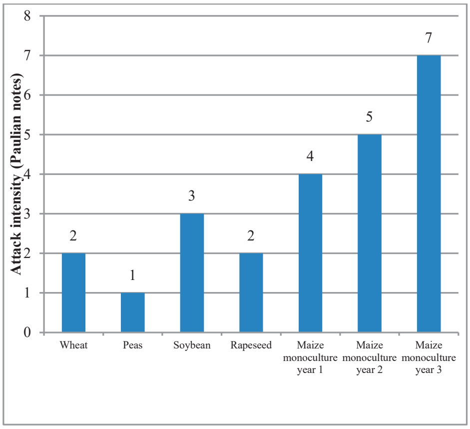

Figure 6. Attack intensity for *T. dilaticollis*

The attack intensity of the *H. armigera* registered the highest percentage of plant attacked in the 3-year maize monoculture (Figure 7). The lowest percentage was recorded for the wheat as preceding crop. Being a polyphagous pest, we can also observe a strong intensity of the attack after the soybean crop, where *H. armigera* started to raise big problems, the value being very close to the maize monoculture year 1.

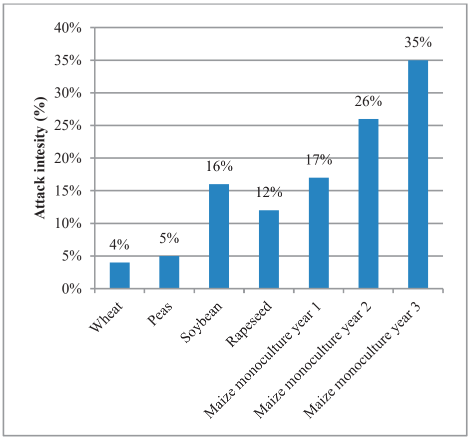

Figure 7. Intensity attack for *H. armigera*

The attack intensity of the *O. nubilalis* registered the highest percentage of plant attacked in the 3-year maize monoculture (Figure 8). The lowest percentage was recorded for the wheat and peas as preceding crops. Although the lowest percentages were recorded for wheat and peas with only 2% of plants attacked, soybean and rapeseed can be considered very good precursor crops as well because the values are very close to the minimum ones.

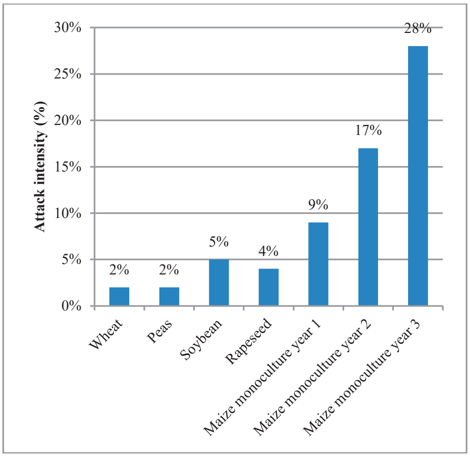

Figure 8. Intensity attack for *O. nubilalis*

CONCLUSIONS

Maize monoculture increase the density and attack intensity of the studied pest, respectively *Tanymecus dilaticollis* Gyll. (maize leaf weevil), *Helicoverpa armigera* (Hübner) (cotton bollworm or corn earworm) and *Ostrinia nubilalis* (Hübner) (corn borer). The values of density and attack intensity are higher the longer the maize monoculture lasts.

The density and attack intensity of *Tanymecus dilaticollis* Gyll. were smallest when the maize followed peas as preceding crop.

The density and attack intensity of *Helicoverpa armigera* (Hübner) were smallest when the maize followed wheat and peas as preceding crops.

The density and attack intensity of *Ostrinia nubilalis* (Hübner) were smallest when the maize followed wheat, peas, soybean and rapeseed as preceding crops.

The crop rotation represents one of the best methods of controlling pest densities and attack intensity in maize crop.

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SUNFLOWER AND SOYBEAN CROPS CULTIVATED IN A MIXED INTERCROPPING SYSTEM, IN THE 2022

Maria JOIŢA-PĂCUREANU[1, 2], Gabriel POPESCU[1], Luxiţa RÎŞNOVEANU[3, 4], Laurenţiu CIORNEI[1], Ancuţa BĂRBIERU[2], Daniela OPREA[2, 5], Gabriel Florin ANTON[2], Claudia DUNĂREANU[6], Victor PETCU[1, 2]

[1]Center for Studies and Research for Agroforestry Biodiversity "David Davidescu" Acad., Romanian Academy, Bucharest
[2]National Agricultural Research and Development Institute, Fundulea, 1 Nicolae Titulescu Street, 915200, Calarasi, Romania
[3]Agricultural Research Development Station Braila, km. 9 Viziru Street, Braila County, Romania
[4]Bucharest University Economic Studies, Faculty of Agri-food & Environmental Economics, Research Center for Sustainable Development, 6 Piata Romana Street, 010374, Bucharest, Romania
[5]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania
[6]Agricultural Research Development Station Simnic, 54 Balcesti Street, Craiova, Dolj, Romania

Corresponding author email: claudia.borleanu@yahoo.com

Abstract

The intercropping system employs growing several species in between each other, during the same season. Intercropping practices differ in arrangement, sowing time, and plant combination. Intercropping has significant advantages over monoculture farming, which aims to boost yields and more efficient usage of land and resources. The most fundamental intercropping benefits include increased profit, better pest management, improved weed management, and enhanced biodiversity and ecological stability. In 2022, an experiment was organized in the intercropping system, using sunflower and soybean crops. Ten sunflower hybrids and ten soybean varieties have been studied, regarding some morphological, and physiological characteristics, quality, and production. The results showed that even though the climatic conditions were not highly favorable for the crops developing, the seed and grain yields released by both crops were very good. There have been some differences between the experimented varieties and hybrids. The best varieties and hybrids, which can be cultivated in this system, can be recommended for the farmers in the studied area.

Key words: *sunflower, soybean, intercropping, advantages, biodiversity.*

INTRODUCTION

The intercropping system employs growing several specie in-between each other during the same season. Intercropping practices differ in the arrangements, sowing time and plant combination.

In intercropping system, the practice is based on principles and rules with respect to different plant families, architecture, time of maturing, sunlight and water (Jeffrey, 2016).

Intercropping has significant advantages over monoculture farming, which are aimed at boosting yields, more efficient usage of land and resources, also a better protection of crops (Runyon et al., 2009; Almeida et al., 2019).

Sunflower is grown in an increasing area in the world (around 29 million hectares), with a production of over 55 million tons and its importance in human and animal nutrition is therefore increasing (Miklic, 2022).

Soybean is economically the most important bean in the world, providing vegetable protein for millions of people and ingredients for hundreds of chemical products (Enciclopedia Britannica, 2023).

Sunflower was reported as a serious candidate for strip, row, and relay intercropping systems (Echarte et al., 2011). Its desirable characteristics, such as erect growth habit, harvestable head, resistance to lodging and drought, and minimal land cover make sunflower an excellent component to intercrop with a short stature and/or duration crop, like soybean (Andrade et al., 2012; De la Fuente et al., 2014). The optimal variety type for successful intercropping has not been extensively examined (Olowe and Adeyemo, 2009). There have been studies on the performance of a small number of sunflower varieties intercropped with soybean (Olowe and Adebimpe, 2009). There have been observed significant differences among the tested varieties.

It must be studied varietal traits for maximizing the performance of the intercropping system.

MATERIALS AND METHODS

It was used a strip intercropping method. This practice involve the arrangement of plants in rows, having six rows each crop, with section of land wide, to facilitate machine operations.

There have been studied nine soybean varieties and ten sunflower hybrids. All of them are commercial varieties.

There have been collected climate data from the Meteorological Station of ARDS Braila.

RESULTS AND DISCUSSIONS

The climatic conditions in Braila location (Figure 1) were not so good, specially regarding the rainfall quantity. It was quite small in all season of plants development. For sunflower the rainfall helped to have quite good development. For soybean it was not sufficient.

The air temperature in time of sunflower flowering was not so high, being favourable, also for soybean.

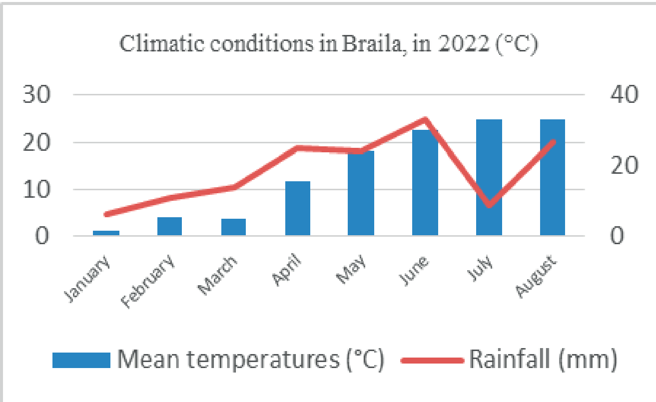

Figure 1. Rainfall (mm) registered in Braila and Fundulea, in year 2022

În Table 1 are presented the results regarding the period from emergence to flowring for the two crops varieties.

For sunflower this period was from 52 to 65 days, for soybean being from 55 to 69 days.

In Table 2 there are presented the results regarding some important characteristics for sunflower hybrids. The highest plant height has the hybrid P64E136 and the lowest NK Brio and Mas 83. The highest head diameter was for the hybrid P64E136, also the TKW value.

The lowest head diameter was for the hybrid Ilinca. The highest HW was in case of the hybrid ES Aver and the lowest for hybrid NK Brio.

Table 1. The period from emergence to flowering, for studied varieties

Variety	The period from emergence to flowering (days)							
	Sunflower				Soybean			
	Int. var.*	Dif.**	Average	CV*** (%)	Int. var.*	Dif.**	Average	CV*** (%)
H1/S1	53-64	11	*57*	6.76	55-64	9	*59*	4.34
H2/S2	53-64	11	*59*	6.58	57-64	7	*60*	4.18
H3/S3	54-65	11	*59*	6.35	58-65	7	*61*	4.36
H4/S4	55-62	7	*58*	4.40	57-65	8	*61*	4.78
H5/S5	54-62	8	*58*	6.03	59-66	7	*62*	4.22
H6/S6	54-62	8	*58*	5.14	57-65	8	*61*	4.90
H7/S7	52-60	8	*56*	5.42	59-67	8	*63*	4.26
H8/S8	55-64	9	*60*	4.89	60-69	9	*64*	4.04
H9/S9	53-61	8	*56*	5.44	57-66	9	*61*	4.77
H10	54-63	9	*58*	6.63				

Table 2. Data regarding some important characteristics, of studied varieties, in Braila, 2022

Sunflower hybrids	Braila			
	Plant height (cm)	Head diameter (cm)	TKW (g)	HW (kg/hl)
Ilinca	123.33	19.67	81.5	43.0
DS 121	127.67	23.67	88.0	43.6
ES Aver	119.50	21.00	80.3	44.7
NK Br	111.33	20.67	80.1	38.0
Mas83	111.33	19.33	85.3	44.9
LG CLP	128.67	22.33	102.4	42.8
P64E136	148.67	23.00	84.3	38.1
FD 27	134.67	22.33	84.6	44.1
FD 70	116.00	20.33	103.5	43.0
Perf.	146.33	20.67	80.7	41.1
Media	126.7	21.9	87.0	42.0

In Table 3 the results are showing some important characteristics for the experimented soybean varieties. The highest plant height was for the variety Andruta and the lowest for Camelia. The highest number of levels with capsules was for the varieties Andruta, Ilaria and Ileana and the highest number of grain in capsule was for the variety Ileana.

Table 3. Data regarding some important characteristics, of studied varieties, Braila, 2022

Soybean varieties	Braila			
	Plant height (cm)	Number of levels with capsules (cm)	Number of capsules on level	Number of grains in capsule
Andruta	95.00	18	4	9
Florina	84.33	12	3	9
Ilaria	93.11	18	4	12
Safta	89.44	16	3	12
Camelia	65.33	14	4	10
Ileana	93.11	18	4	14
Iris TD	68.11	12	4	8
Turda	80.00	16	3	8
Adonis	78.00	14	4	12

The results in Table 4 show that the resistance to lodging and to stem broken is very good for all hybrids, also, the resistance/tolerance to some important diseases is very good. Very important is the behavior of the sunflower hybrids regarding the resistance to the parasite *Orobanche cumana* (broomrape). Even the hybrids which have not a good genetic resiatance to the parasite had a small attack degree. It must be mentioned that the hybrids having resistance to imazamox (CL or CLP) have not been trated with herbicides.

Table 4. Some characteristics of sunflower hybrids, in Braila area, in 2022

Sunflower hybrids	Braila			
	Resistance to stem broken (notes)	Resistance to lodging (notes)	Resistance *Phomopsis* (%)	Resistance *Orobanche* (%)
Ilinca	2	1	5.9	6.7
DS 121	2	1	2.1	3.9
ES Aver	1	1	0.8	3.5
NK Brio	2	2	1.8	3.8
Mas83	1	1	6.3	6.2
LG CLP	1	1	3.6	4.7
P64136	2	2	1.7	0.4
FD 27E	1	1	2.3	1.2
FD70CL	1	1	3.7	4.2
Perf.	1	2	8.4	21.6

In Figure 2 are presented the results regarding the seed yield, for sunflower hybrids experimented in Braila. The highest seed yield was released by LG 58390 CLP, ES Averon SU and FD15E27 hybrids.

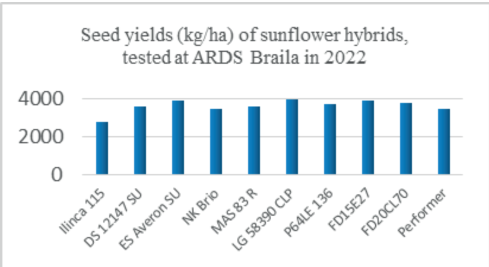

Figure 2. Seed yields of sunflower hybrids tested in Braila

In Figure 3 are presented results regarding the grain yield for soybean varieties, studied in an intercropping system with sunflower. The highest yield was released by Ileana, Ilaria, and Safta varieties.

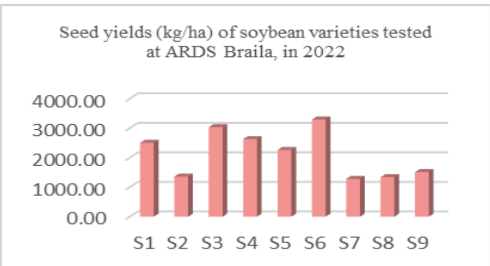

Figure 3. Seed yields of soybean varieties tested in Braila

CONCLUSIONS

The studied sunflower hybrids and soybean varieties have different vegetation period, depending by variety genetic, also by the climatic conditions, specific to the location of cultivation.

Even the climatic conditions in the experimentation period were not as it is request for these crops, this system of cultivation bring benefit for each sunflower and soybean.

The sunflower hybrids, cultivated in this system, have good resistance to lodging and stem broken, due to the genotype, also to the cultivation system. Also, very good behavior had the hybrids regarding the resistance/tolerance to the pathogen *Phomopsis helianthi* and to the parasite broomrape.

The grain yield was very good, for many of studied varieties, in this type of cultivation system.

We consider that, among the protection of the crops by some pathogens or pests, this system of cultivation was given the attractivness for some beneficial insects.

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